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Agilent Technologies

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2700 Mitchell Drive
Walnut Creek, CA 94598-1675/USA

500-MS IT Mass Spectrometer
MS Workstation
Version 6

Software Operation Manual

QUALITY SYSTEM ISO 9001 CERTIFIED

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Introduction

Overview

Varian MS Workstation Version 6.9 controls the Varian 500-MS, associated Varian chromatography modules, and the LC Combi PAL AutoSampler. Use the workstation to manage MS hardware, and data acquisition, display, reduction, and analysis.

This manual in association with the 500-MS Hardware manual, the MS Software Reference Manual, and the manuals for each of your LC modules, and AutoSamplers provides you with the essential information you need.

As you start up your system and begin acquiring data with it, you interact with a window that simplifies your tasks for controlling the 500-MS system. As you build methods, edit, and analyze acquired data, you will notice that the interface has the versatility and power needed for complex mass spectrum and chromatography data handling.

This Operation Manual is organized to help you understand your instrument quickly. After the introduction, applications that compose the Varian MS Workstation are outline. Read this section to get an overview of the Workstation structure and operation. The sections following the Workstation Overview outline how to do manual injections and automated injections.

If you plan to move your system or change its configuration, read the sections on installing the software and configuring your system. The manual for qualitative and quantitative analysis is MS Workstation Software Reference Manual part number 0391496300. Explore many features and advantages of this software.

Additional Manuals

The documentation for the 500-MS includes the following manuals. Before using the system, refer to the following manuals on the Varian MS Workstation CD. Read all instructions thoroughly.

500-MS Hardware Operation Manual

This provides information for installing, maintaining, using, and repairing your 500-MS System. All of the information included in this manual is also available in the Online Help section of the software, part number 0395407600.

MS Workstation Software Reference Manual

This manual provides a practical way to learn quickly how to perform basic tasks using the MS Workstation Software. These operations include displaying and reviewing chromatographic and mass spectral data, library searching, and quantitative analysis, part number 0391496300.

MS Data Handling Users Guide

This manual contains post acquisition Data Handling procedures and tasks. This manual can help you develop and select the best type of Data Handling for your method, part number 0395403800.

MS Workstation Tutorial Manual

This manual demonstrates the MS Workstation capabilities in tutorial formats. While these exercises use data files from the Saturn 2000 ion trap, they illustrate the principles of the Varian WS software and can be adapted to the Varian 500-MS, part number 0391498800.

Data Acquisition with LC Control

This manual describes the operation of the MS Workstation with Varian HPLC modules. It covers configuring different modules for data acquisition, building a method, and running samples, part number 0391473200.

Other Manuals

Please refer to the manual(s) of your other instruments.

Valuable information on operation as well as cautions and notes are contained within these manuals.

The primary interface between you and your 500-MS is through the PC hosting your Varian MS Workstation. Please refer to these manuals first when you need information regarding a task or a feature of the system.

Before Using Your System

1. Is the system turned on and ready? If not, refer to the 500-MS Hardware Operation Manual.
2. Do you need to tune the mass spectrometer? Refer to the 500-MS Hardware Operation Manual and the Autotune section in this manual.
3. Prepare your samples.
4. Do you have methods built for both 500-MS and chromatography modules? If not, refer to the 500-MS Method section of this manual and to the manuals for your chromatography devices such as LC pumps and Autosampler.
5. Perform the injection. Refer to “Injecting Samples” on page 96.
6. Review your data. Refer the MS Workstation Software Reference Manual.

MS Workstation Applications

Overview

The Varian MS Workstation is a series of applications that are displayed on the Workstation toolbar. The tool bar can be displayed as a series of large buttons.

Or as a series of small buttons.

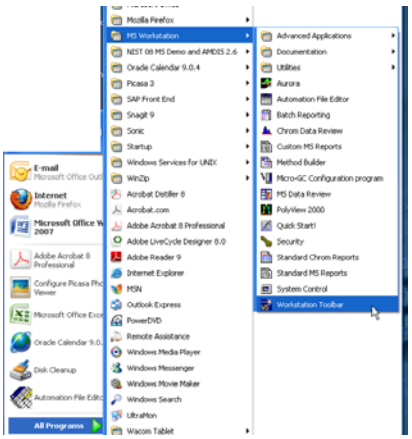
Tooltips show the application name when the cursor rests on the Application Icon.

Application Descriptions give brief descriptions of the application that will be opened when the cursor rests on the Application Button.

Quick Link Buttons provide menu selections of operations to be performed on the listed file.

When activated, the Workstation toolbar is like the Windows taskbar. It can be docked on any of the four sides of the display screen and other Windows programs do not cover or go behind it when they are opened in full screen mode.

If the Workstation toolbar is not already opened, open it from the Windows Start Menu as follows:



Application Buttons

System Control/ Automation		Monitor instrument status, and perform automated injections and batch recalculations.
View/Edit Methods		View and edit instrument operation, data acquisition, and data handling methods.
Edit Automation Files		Do off-line editing of SampleLists, RecalcLists, and SequenceLists.
Review/Process MS Data		Review chromatograms and spectra, perform library searches, and review and process quantitation results.
Standard MS Reports		Create, edit, and view standard MS reports.
Custom MS Reports		Create, edit, and view customized MS reports.
Security Administration		Set 4000 GC/MS Workstation security options and passwords.
SMS/MS File Conversion		Convert data files between DOS and Windows formats.
DAT to XMS		Convert Kodiak Dat files to MS Workstation xms format.
Batch Reporting		Generate standard reports for a group of Data Files by dragging and dropping them on the Batch Report Window.
View/Edit Chromatograms		Review standard GC chromatograms, interactively edit data handling parameters, and recalculate results.
Standard Chrom Reports		Preview standard chromatogram and results reports.
Compound Set Editor		Create or edit sets of compounds in the MS Data Handling method. This allows activation/deactivation under automation.
PolyView™2000		View and process PDA data from your Varian HPLC Photo Diode Array detector.
Aurora PDA		Use Aurora to view HPLC Photo Diode Array data in a 3D presentation.
Quick Start		Run a sample without a Sample List.

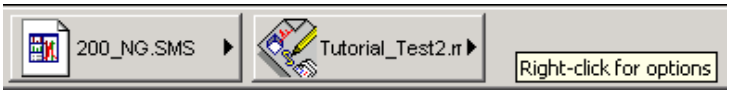
Workstation Toolbar Options

Moving the Workstation Toolbar

The Workstation toolbar can be moved to any edge of the Windows screen. Click a portion of the Workstation Toolbar that does not contain Application or QuickLink buttons and drag the toolbar to the edge of the desired screen.

When you release the mouse, the toolbar remains on that edge. The Workstation Toolbar will open to this location the next time it is started.

The Workstation Toolbar Options Menu

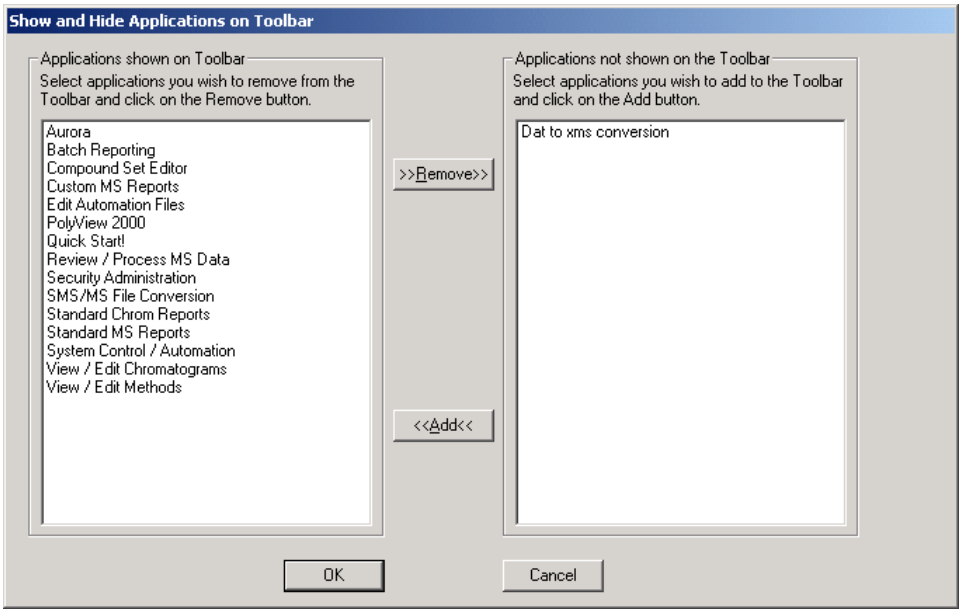


When you move the mouse over an area of the Workstation Toolbar not containing application or QuickLink buttons, you may right-click to display an option menu.

Move to Windows Taskbar

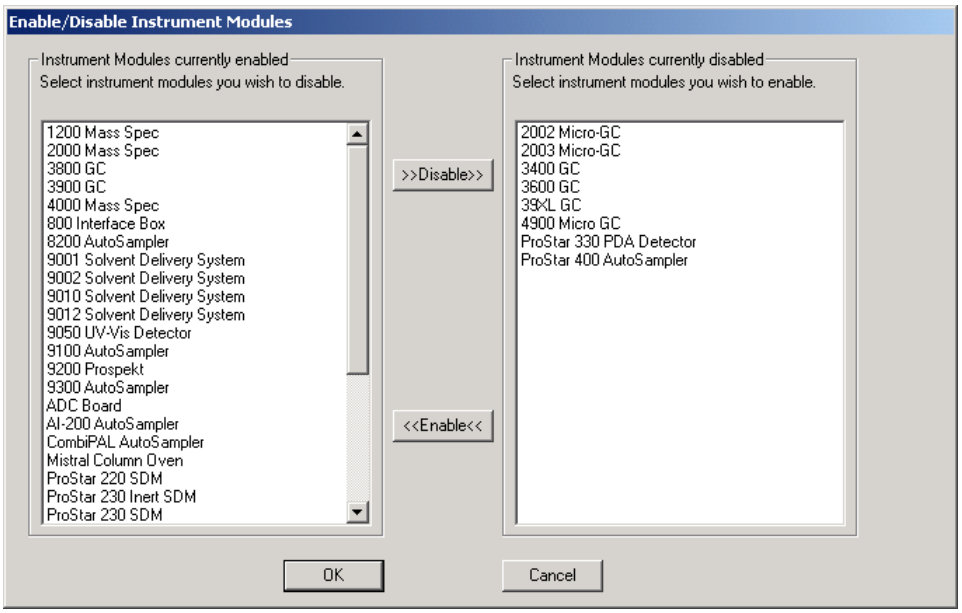
You can display the Workstation toolbar as a Windows taskbar icon. Select **Move to Windows Taskbar** from the Workstation toolbar options menu. Taskbar icons appear in the lower right (or bottom) of the Windows taskbar, (the bar on which the **Start** button appears). Click the **Workstation Toolbar** icon to display the options menu.

Show/Hide Applications on Toolbar



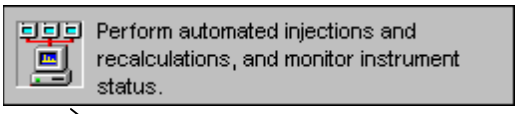
Select which MS Workstation applications are represented by icons on the Workstation toolbar. The list on the left shows the applications that are currently displayed. The list on the right shows applications that are installed but not displayed in the toolbar. To remove an icon from the toolbar, select it from the left list and click **Remove**. To add an icon to the toolbar, select it from the right list and click **Add**.

Enable/Disable Instrument Modules



Select which MS Workstation instrument modules are available. When available, the module appears in System Control if the corresponding instrument is connected and turned on. The left list shows the instrument modules currently installed and enabled in the MS Workstation. The right list shows the instrument modules installed but not enabled. To disable an instrument module, select it from the left list and click **Disable**. To enable an instrument module, select it from the right list and click **Enable**.

Application Descriptions



Application Descriptions are a brief description that appears when the cursor rests on the application button

When this menu item is checked, application descriptions are displayed when you move the mouse over application buttons in the Workstation Toolbar.

Small Buttons on Toolbar

When this menu item is checked, the Workstation Toolbar is reduced in size.

Run Application

Lists the applications on the Workstation toolbar. Select an item from this list, to launch the application.

Help on

Lists all applications on the Workstation toolbar. Select an item from this list, to display online help.

Pick Data File for Quick Link Button

Lists the eight most recently used Data Files in order of use. Select a Data File from this list, to display it in the Quick Link button.

Pick Method for Quick Link Button

Lists the eight most recently used Methods in order of use. Select a Method from this list, to display it in the Quick Link button.

Help on Workstation Toolbar

Displays the help currently in use.

Product Support Web Site

If you have Internet access and a web browser installed on your computer, this automatically opens the MS Workstation Product Support Web Site. Here you will

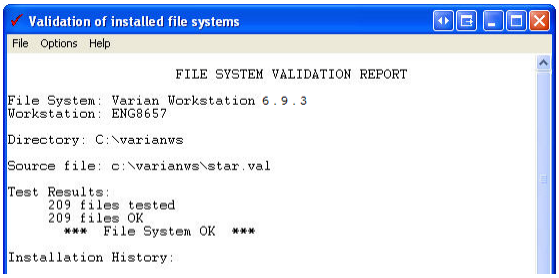
find the latest software and documentation updates for the MS Workstation suite of products, along with additional notes, tips, and answers to frequently asked questions.

Visit this site periodically for new information.

About Workstation Toolbar

The About window has information about the MS Workstation software version, installation history, and a list of the instrument control modules installed. The following is an example.

The **Validate Installed Files** uses checksums produced at the factory to test and document errors. You can print a report that documents the file system tested, the success or failure of the test, the date printed & tested, the ID of the tester (if login IDs are used on this computer). The report can contain a signature line. For more details about this application, refer to the online help. The following is an example.



Quit

This menu item closes the Workstation Toolbar application. If you choose to run the Workstation Toolbar automatically when Windows starts, the Workstation Toolbar opens the next time you start Windows.

Launching Applications from the Workstation Toolbar

Using the Application Icon Buttons

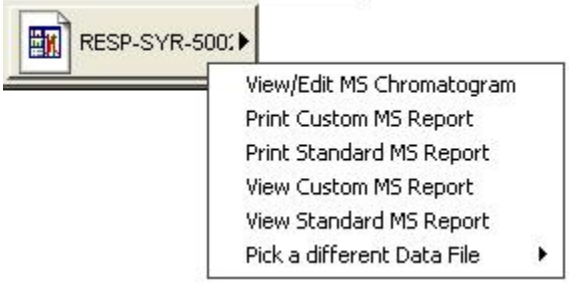
The MS Workstation applications are icons on the Workstation toolbar. When you pause the cursor on the icon, the name of the application is displayed in a tooltip below the cursor and on the right of the toolbar, a description is displayed.

Click an icon in the Workstation toolbar to launch the corresponding application.

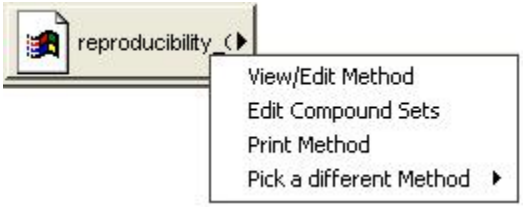
Using the Data File and Method QuickLink Buttons

The two buttons to the right of applications are QuickLink to Data Files and Methods.

Data Files: Display a menu of options for the most recently used Data File.



Methods: Click to display operations that can be performed on the most recently used Method.

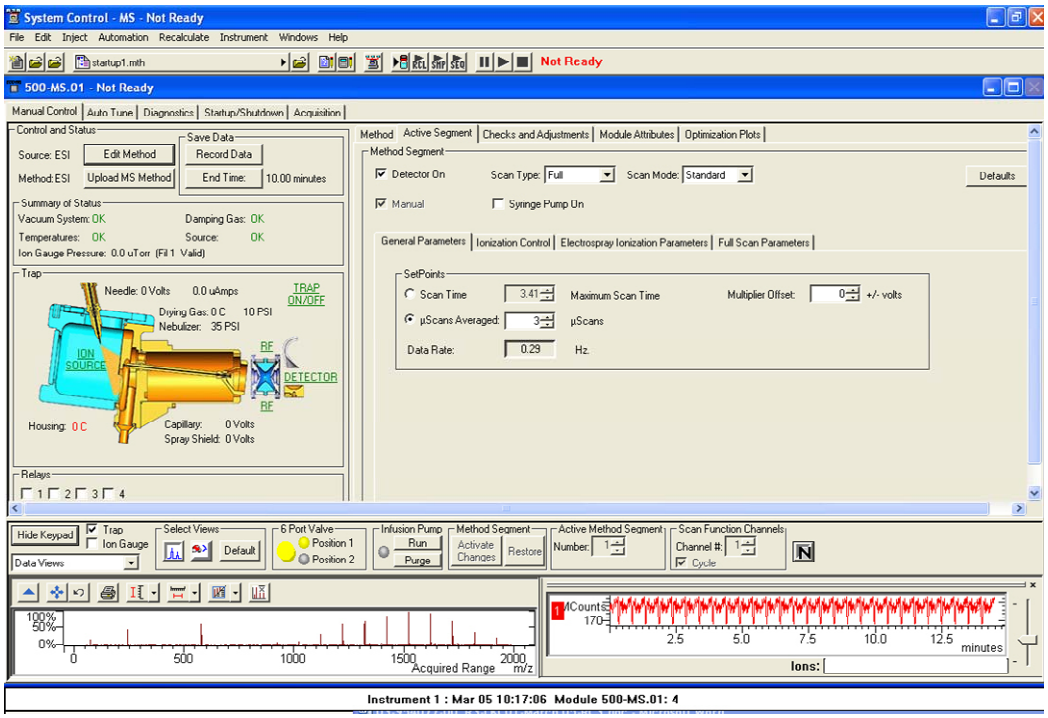


System Control

Overview

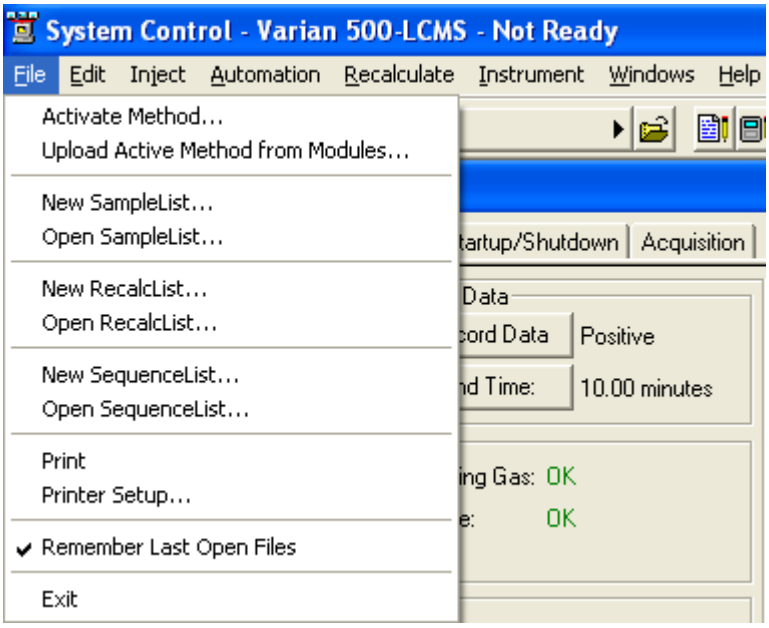
This section introduces the features of System Control. Later sections explain how to use these features.

Navigating the Instrument Window



System Control features include System Control Menu bar, System Control Toolbar, 500-MS Keypad Dialog, Relays, Chromatogram/ Spectrum/ Optimization Plot Toolbars, and Chromatogram/Spectrum/ Optimization Plot Displays.

File Menu



Activate Method: Open an existing method.

Upload Active Method from Modules: Save conditions of an existing module to a new or existing module. This method becomes the active method with the conditions of the active modules.

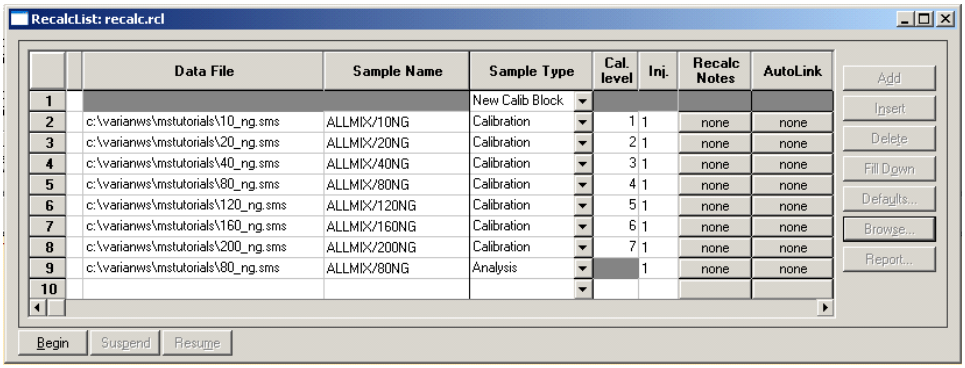
New SampleList: Create a SampleList.

Open SampleList: Open an existing SampleList..

New RecalcList: Create a RecalcList.

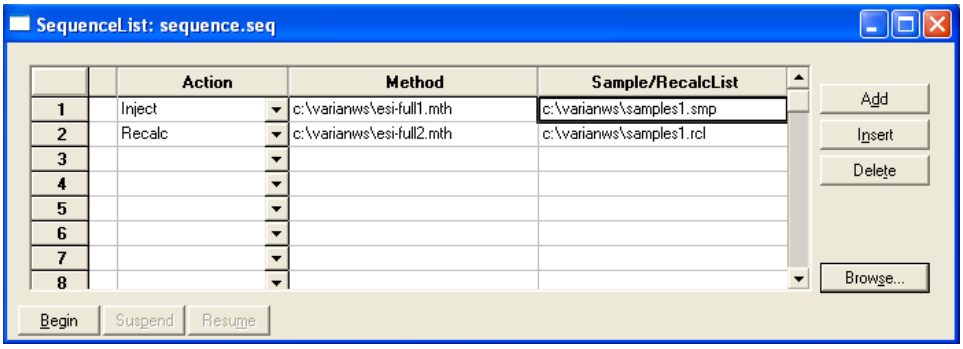
Open RecalcList: Open an existing RecalcList. The RecalcList dialog box follows:

NOTE: Process a RecalcList either in System Control (by clicking **Begin**) or in MS Data Review. In MS Data Review, use Process Data to access this feature.



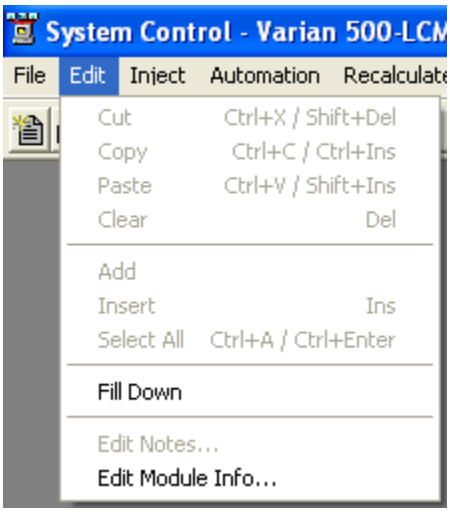
New SequenceList: Create a SequenceList.

Open SequenceList: Open an existing SequenceList. The SequenceList dialog box follows.

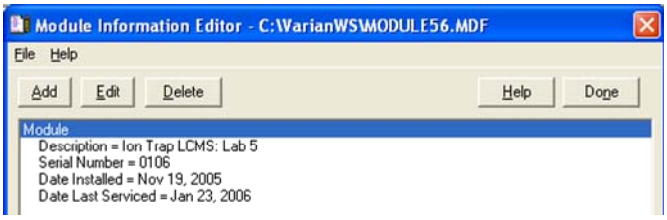


Edit Menu

Click **Edit** and select **Edit Module Info**.

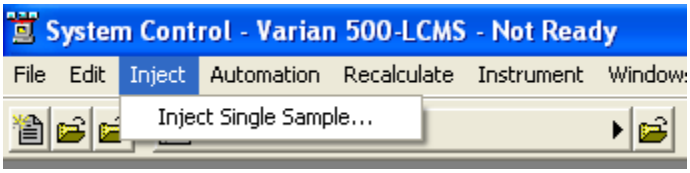


Each hardware module in your system (212-LC, 500-MS, Combi PAL AutoSampler, etc.) contains its own message log and documentation screen. Use the Module Information Editor to keep a record of performance, maintenance, hours used, or other comments.

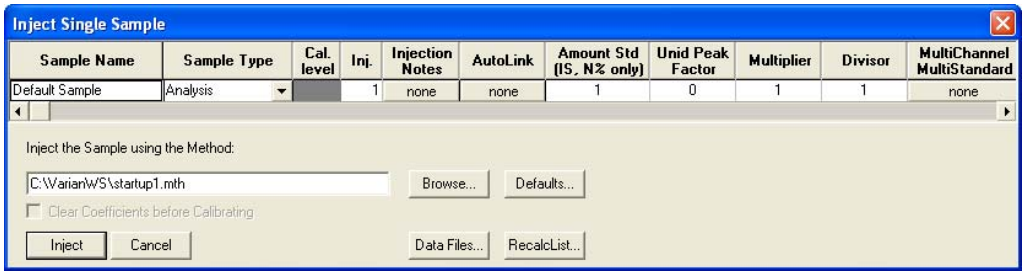


Use **Add**, **Edit**, **Help,** and **Delete** to assist you. Click **Done** when done.

Inject Single Sample Menu

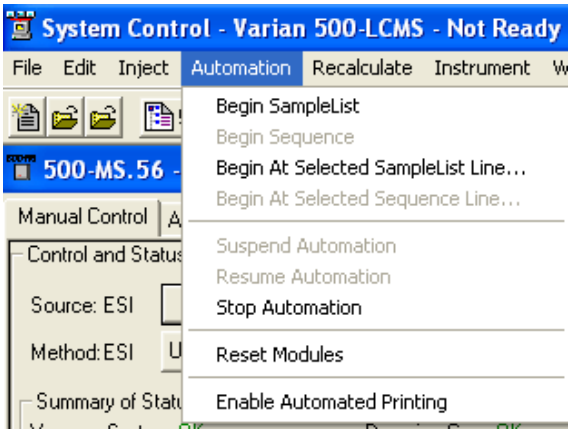


Inject Single Sample opens a one line SampleList. The following dialog box appears when this command is invoked. Click **Inject** (bottom left corner) to inject the sample by manual injection, infusion, or autosampler. The 500-MS automatically switches to Acquisition mode. See “Injecting Samples” on page 96.



Automation Menu

The Automation menu list.



Begin SampleList: Automation starts at line 1 and ends at the last line of the active SampleList. Open SampleList from the File menu to use this feature.

Begin Sequence: Change methods and SampleLists during automation. A Sequence must be open from the File menu to use this feature.

Begin At: Starts automation at a particular Sequence or SampleList line and runs until the last line is completed. A SampleList or SequenceList must be open to use this feature.

Suspend Automation: Stops automation at the last completed sample.

Resume Automation: Starts automation after it has been suspended. Automation starts from where it was suspended.

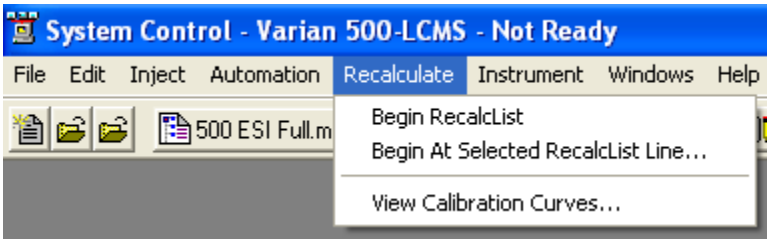
Stop Automation: Ends automation immediately and resets all modules.

Reset Modules: Brings the various modules to their ready states.

Enable Automated Printing –Print reports during automation.

See “Injecting Samples” on page 96 for more information.

Recalculate Menu

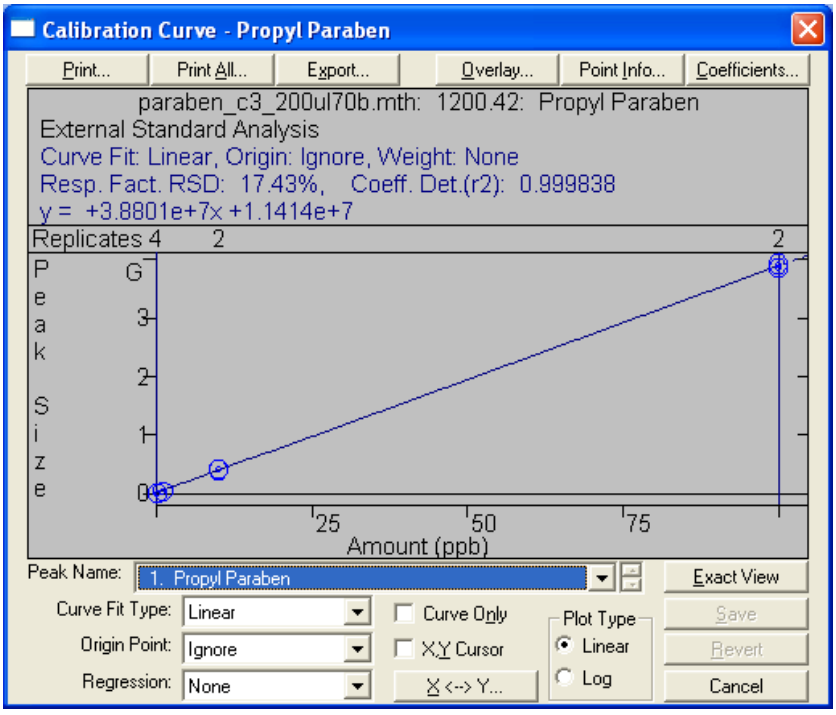


Begin RecalcList: Start an automated recalculation. A RecalcList must already be prepared and opened under the File menu or a new RecalcList will need to be made.

Begin at Selected RecalcList Line: Process a RecalcList starting with a selected line and proceeding with the rest of the list.

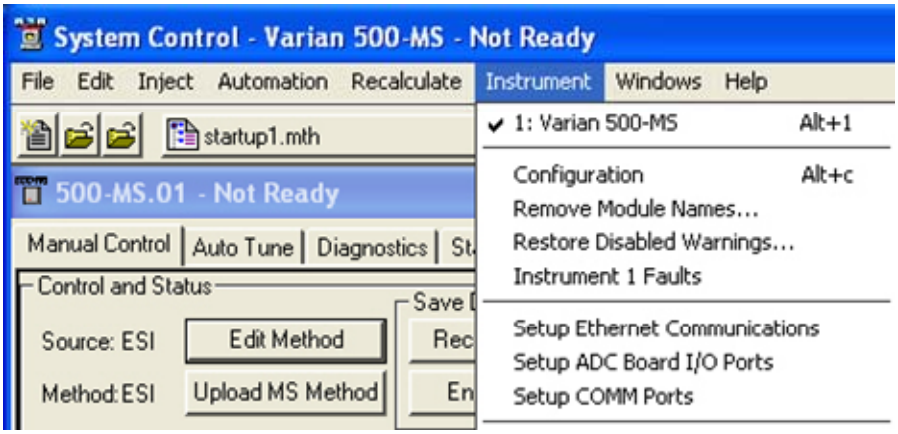
View Calibration Curves: View any curve for the calibrated compounds in the active method. An example of the calibration curve screen follows. For a complete explanation of this screen, go to the Quantitation section.

NOTE: Recalculation may be performed in System Control or in MS Data Review from the ***Process Data*** icon.



Instrument Menu

The Instrument menu follows.



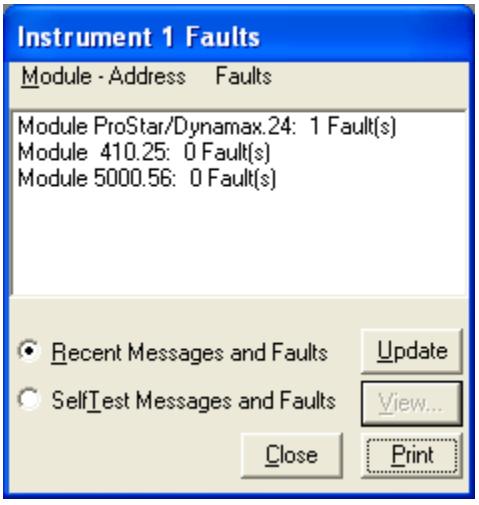
Varian 500-MS: The configured instrument.

Configuration: Add new modules to the existing instrument. This is necessary if additional hardware is added such as a Combi PAL AutoSampler.

Remove Module Names: Remove the association between a Module Name and a Module Address. You can connect a Module with a different Module Name at that address. The next time the associated Module connects at that address, you are prompted to select the correct name for the Module.

Restore Disabled Warnings: Enable warning message you disabled.

Instrument 1 Faults: See if there are faults in the modules in Instrument 1 except for the 500-MS. An example follows. Use the update key to check for new faults without opening dialog. The 500-MS error messages are displayed in the Message Log.

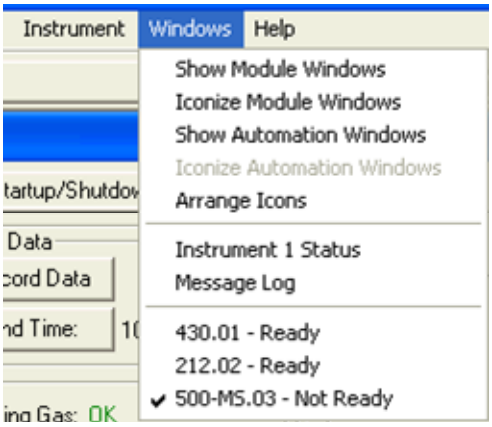


Setup Ethernet Communication: Set up communication between an Ethernet controlled device, such as a 335 photodiode array detector, and System Control.

Setup COMM Ports: Set up communication between a COMM Port controlled device, such as the ProStar 430 Autosampler, and System Control.

Windows Menu

View the Windows associated with System Control, see the following.



Show Module Windows: View the Module Windows configured in the instrument. In this example, the Modules available are the 212-LC pump, 430 Autosampler, and the 500-MS.

Iconize Module Windows: Change the individual Module Windows to icons at the bottom of your screen.

Show Automation Windows: View the automation screens currently open. These could be the SampleList, RecalcList, and SequenceList.

Iconize Automation Windows Change any open automation window into an icon.

Arrange Icons: Arrange the icons in a row at the bottom of the System Control window.

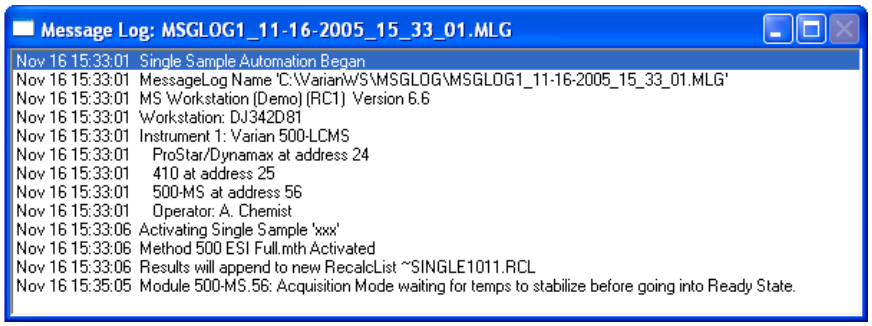
SequenceList: Display the active SequenceList.

SampleList: Display the active SampleList.

RecalcList: Display the active RecalcList.

Instrument 1 Status: View the status of the modules connected to the system.

Message Log: View the event log for the MS module. A sample log display is shown. The error messages of the 500-MS Module are displayed.


Message Log: MSGLOG1_11-16-2005_15_33_01.MLG
Nov 16 15:33:01 Single Sample Automation Began
Nov 16 15:33:01 MessageLog Name 'C:\VarianWS\MSGLOG\MSGLOG1_11-16-2005_15_33_01.MLG'
Nov 16 15:33:01 MS Workstation (Demo) (RC1) Version 6.6
Nov 16 15:33:01 Workstation: DJ342D81
Nov 16 15:33:01 Instrument 1: Varian 500-LCMS
Nov 16 15:33:01 ProStar/Dynamax at address 24
Nov 16 15:33:01 410 at address 25
Nov 16 15:33:01 500-MS at address 56
Nov 16 15:33:01 Operator: A. Chemist
Nov 16 15:33:06 Activating Single Sample 'xxx'
Nov 16 15:33:06 Method 500 ESI Full.mth Activated
Nov 16 15:33:06 Results will append to new RecalcList ~SINGLE1011.RCL
Nov 16 15:35:05 Module 500-MS.56: Acquisition Mode waiting for temps to stabilize before going into Ready State.

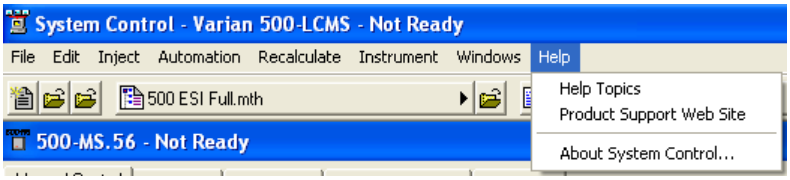
430.01: Display the 430 Autosampler module control screen.

212.02: Display the 212-LC module control screen.

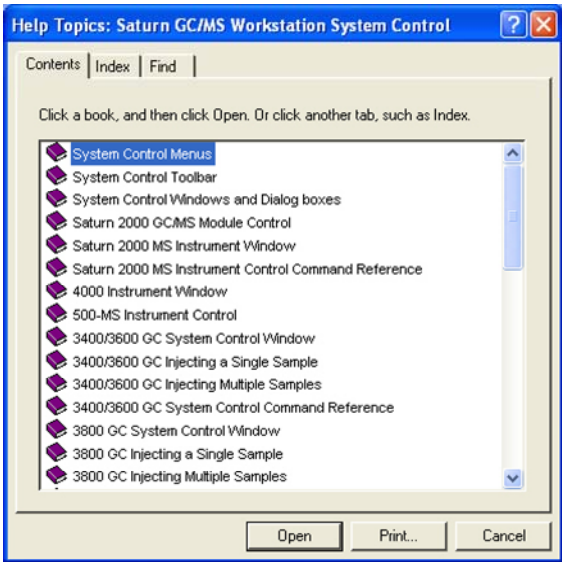
500-MS.03: Display the 500-MS module control screen.

Help Menu

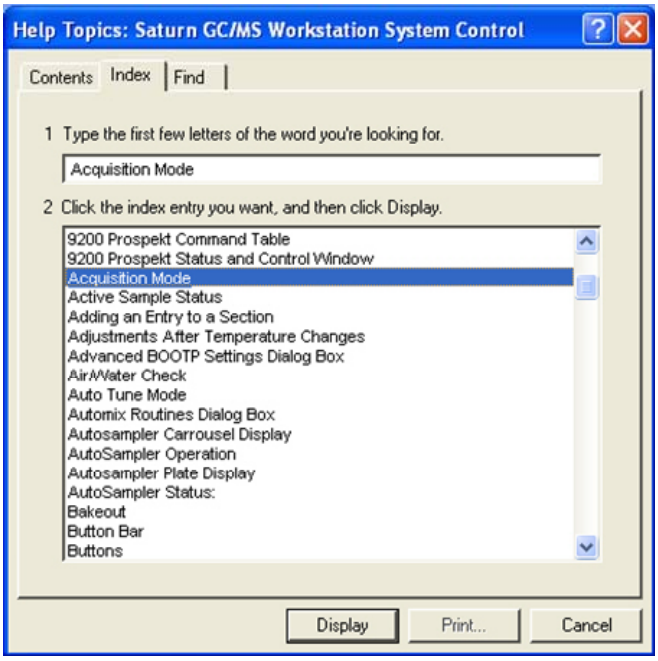
1. Click **Help** on the **System Control** menu bar.


System Control - Varian 500-LCMS - Not Ready
File Edit Inject Automation Recalculate Instrument Windows Help
500 ESI Full.mth
500-MS.56 - Not Ready
Help Topics
Product Support Web Site
About System Control...

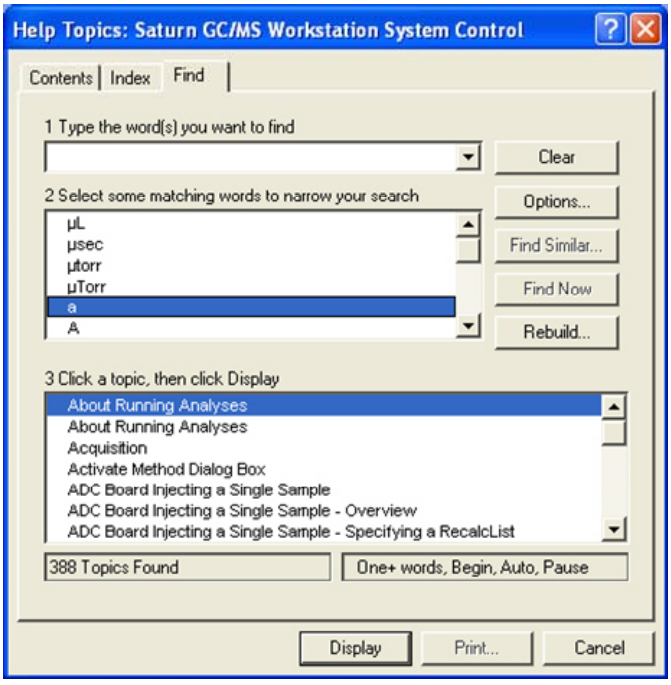
2. Click Help Topics to display the following. Click an item in the Contents box.


Help Topics: Saturn GC/MS Workstation System Control
Contents
Index
Find
Click a book, and then click Open. Or click another tab, such as Index.
System Control Menus
System Control Toolbar
System Control Windows and Dialog boxes
Saturn 2000 GC/MS Module Control
Saturn 2000 MS Instrument Window
Saturn 2000 MS Instrument Control Command Reference
4000 Instrument Window
500-MS Instrument Control
3400/3600 GC System Control Window
3400/3600 GC Injecting a Single Sample
3400/3600 GC Injecting Multiple Samples
3400/3600 GC System Control Command Reference
3800 GC System Control Window
3800 GC Injecting a Single Sample
3800 GC Injecting Multiple Samples
Open
Print...
Cancel

3. Click Index, select a topic, and click Display.



4. To use Find, enter a key word or phrase.



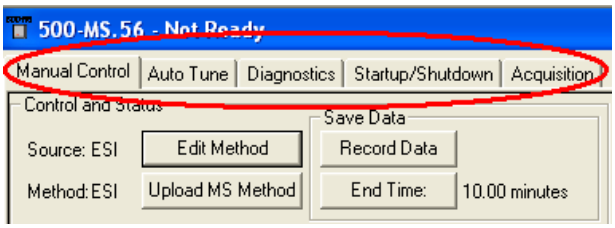
System Control Toolbar

The system control toolbar contains the following functions.

	Create a new automation file.
	The first icon opens an existing automation file.
	The second icon opens the Message Log file.
	View/edit or print a Method
	Open a Method
	Edit notes for an automation file.
	Edit information for an online Module
	Open Instrument 1 Status
	Inject a single sample.
	Start an open RecalcList.
	Start an open SampleList.
	Start an open SequenceList
	Suspend a running list.
	Begin a list
	Stop a running list.

Control Module Window

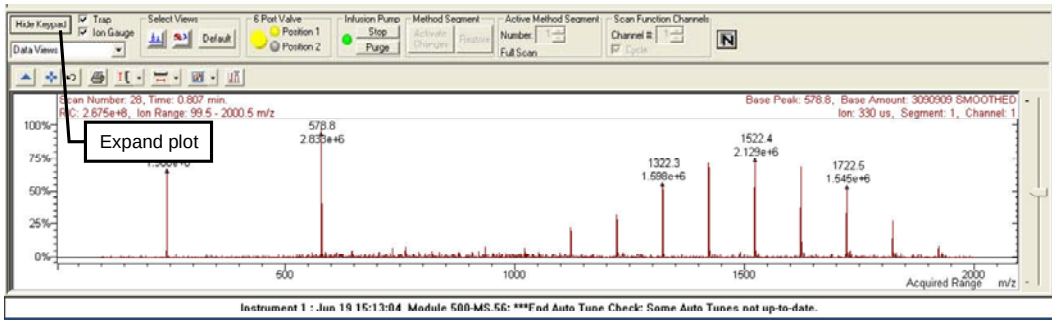
The 500-MS Control Module Window has five views: Manual Control, Auto Tune, Diagnostics, Startup/Shutdown, and Acquisition.

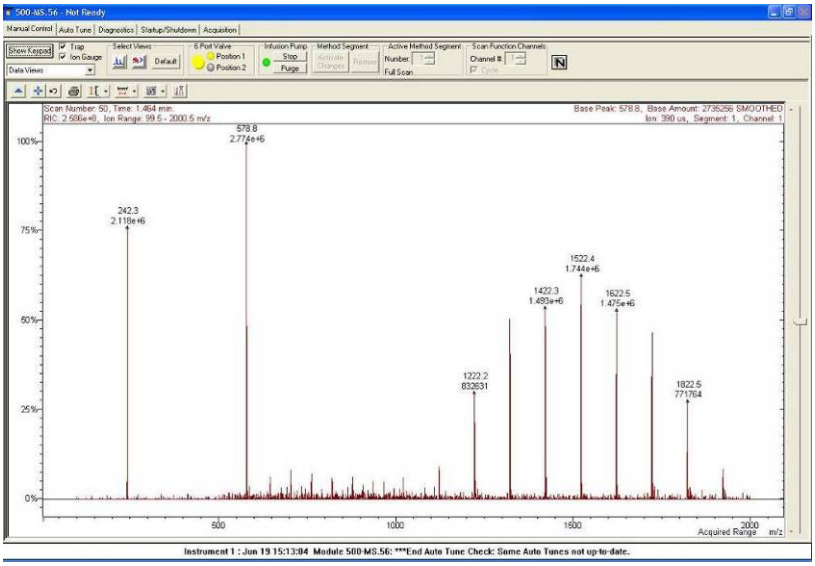


For more information, see the appropriate section.

500-MS Keypad

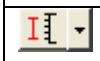
Click Hide Keypad to expand the plot area. The button name becomes Show Keypad. Click it to restore the keypad section.





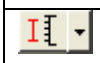
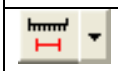
Chromatogram Toolbar

There is a Chromatogram window in Acquisition and in Manual Control. The Chromatogram toolbar for each is slightly different.

	Click Arrow to hide the toolbar. Click in the upper left of the display to restore the toolbar.
	Click Full-Scale to normalize the chromatogram vertically and horizontally. Double-click the lower left corner to do the same.
	Click Previous-Scale to return the chromatogram display to the scaling prior to the current one. Click twice to step through the
	Click Scale to switch between Auto Scale and Fixed Mode Intensity.
	Click Multiple Plots View to select a display.
	Click Spectrum Select to display spectra from the current chromatogram. If not selected, the spectral display is updated
	Click Chromatogram Viewer to load the data file being acquired into MS Data Review to do qualitative analysis in real time, and print results. Available in Acquisition only
	Click Clear Plot to clear the window. Only available in the Chromatogram Plot window in Manual Control.
	Click to display a plot of the Intensity of the Base Peak. To display the RIC trace and the Intensity of Base Peak plot at same time, enter IBP in the Ions field. Clicking the Base Peak icon does not affect the display of the extracted ion chromatogram.

Spectrum Toolbar

View Spectrum display when the 500-MS module is in Manual Control, Auto Tune, or Acquisition modes. The Spectrum Toolbar is slightly different for Manual Control

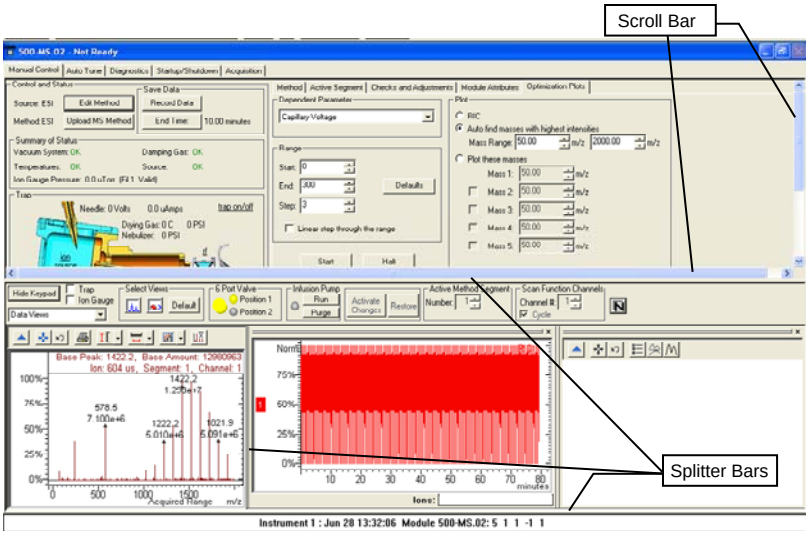
	Click Arrow to hide the toolbar. Click the upper left of the display to restore the toolbar.
	Click Full-Scale to normalize the spectrum vertically and horizontally. Double-click the lower left corner to do the same.
	Click Previous-Scale to return the display to the prior scaling. Click twice to step through the previous displays.
	Click Printer to preview the spectrum report before printing
	Click Scale to switch between Auto Scale and Fixed Mode Intensity.
	Click Mass Range to select Acquired, Fixed or Maximum Mass Range.
	Click Display Type to select between graphical and text displays of mass spectra or display additional information
	Click Average Spectra to average every 10 spectra and apply this average to the real-time chromatogram display. This is not used when recording data. Not available in Acquisition Mode.

Manual Control Mode

The Manual Control Window is divided into two panes, the 500-MS Keypad above and the Data Views/Module Attributes display below. If the screen resolution is not set properly, not all of the features may be displayed.

Configuring the Display

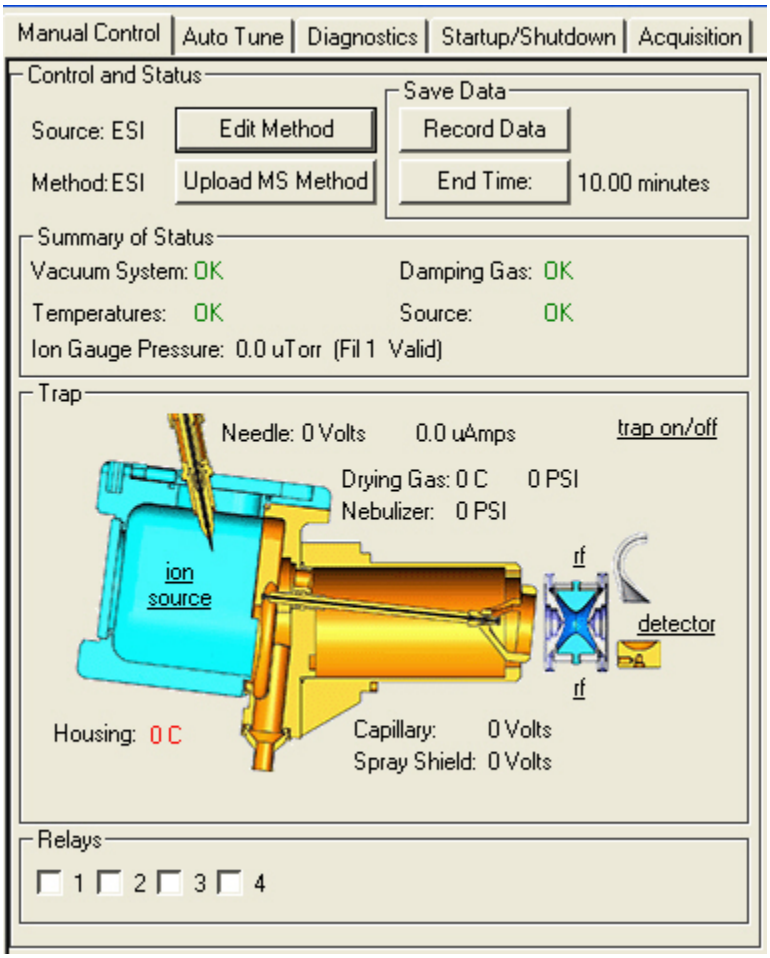
Slide the Scroll Bar, on the right of the Keypad, to view the lower section of the Keypad or slide the Splitter Bar to expand the viewing area of the Keypad. Click and drag a window pane to expand and move it.



Control and Status

The 500-MS Trap Icon

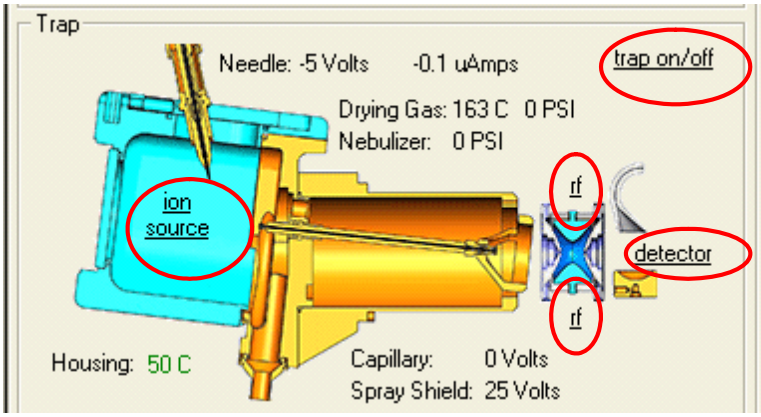
A graphic picture of the trap, on the left, displays the status. When the ion trap is off, most of the text is black indicating that the ion trap is off. The spray chamber housing temperature is always on and green.



The ion trap turns off when there is an instrument fault. Clicking the underlined text turns on that part of the ion trap system.

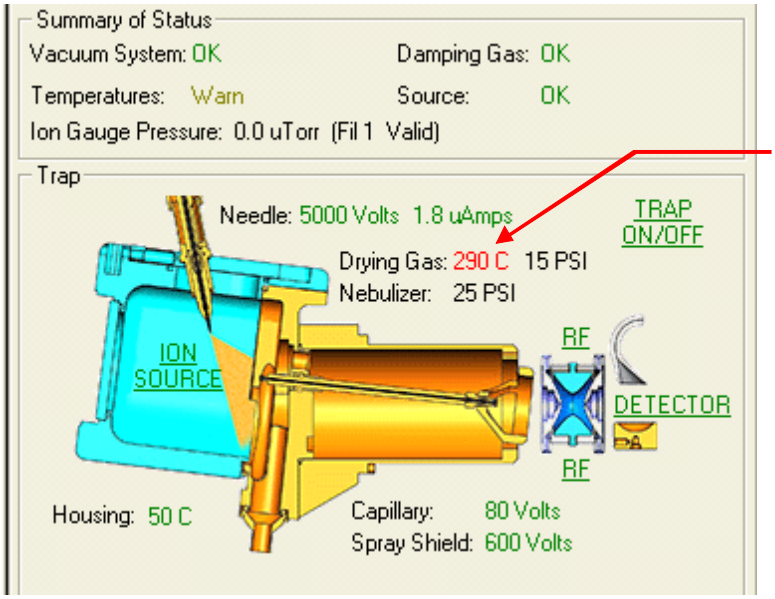
Off -- underlined text is black and lower case,

On -- underlined text is green and in capitals



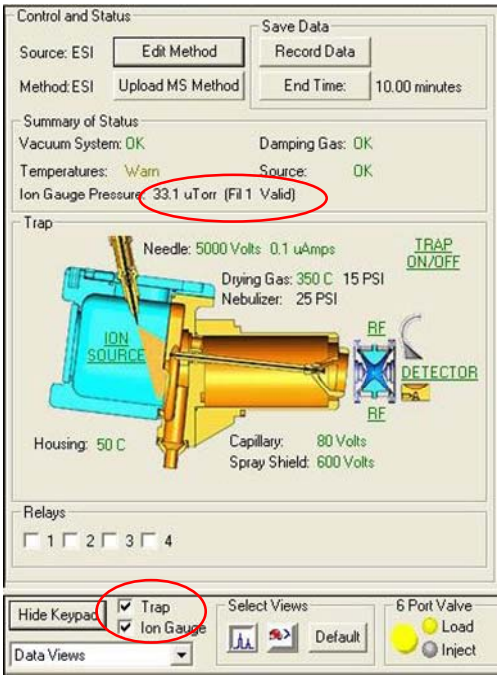
Click trap on/off to turn on the entire trapping system.

Click ion source (Nebulizer & Drying gases and needle voltage), rf, and detector (multiplier) text to turn on those functions. The appropriate fields turn green when ready. This indicates that the ion trap is on, as displayed next.

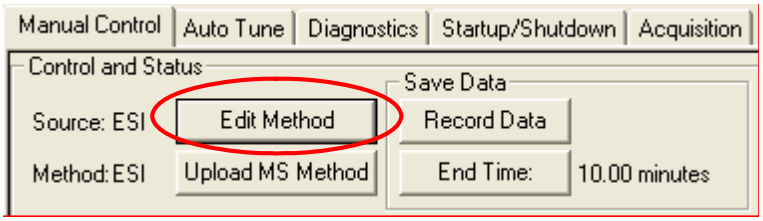


Summary of Status

Green type indicates OK and red indicates a problem or a read back.
Display the vacuum pressure by checking the **Ion Gauge** checkbox next to Hide Keypad.

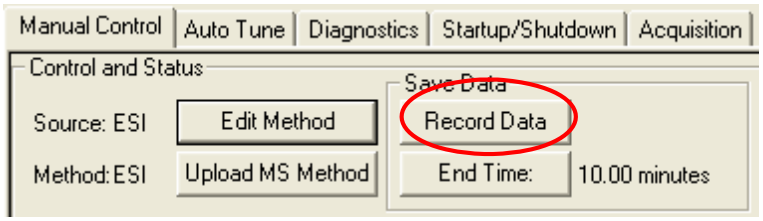


Edit/Upload Method



Click Edit Method to load the current active Method into Method Builder. Click Upload MS Method to create a new Method. You are prompted to save it with the current manual control settings and make it the active Method.

Save Data



Click Record Data to begin data collection using the parameters in the current active Method segment. During data collection, you can switch to different Method segments, and change individual Method segment parameters to observe and record the data. Click End Time to increase the run time if necessary.

When the End Time is reached or when the Stop Acquisition is pressed, the Create Data File dialog appears and you can specify a file name and a location. The data file contains only raw data, so no log information is stored.

NOTE: The Record Data operation does not operate the hardware. It does not automatically turn on the trap, switch the 6 Port valve, start or stop the infusion pump, etc. You must do these operations before, during, or after data collection.

Relays

In **Manual Control**, click to select a relay. Set up Relays in Special Applications in the Method Builder. See the special applications section of the Method Builder for information about how to set up the Relays.

Quick Control Toolbar

The Quick Control Toolbar has controls for the method, hardware, and data.

Method Controls

Activate Changes: Click to activate a method change.

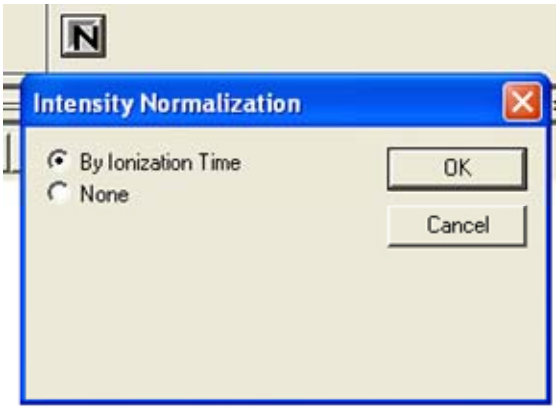
Restore: Click to Restore a previous method.

Active Method Segment: Scroll to active individual method segments.

Full Scan: Displays scan range while acquiring data.

Scan Function Channels: Select a scan channel or cycle through them.

: Intensity Normalization: Click to normalize intensity By Ionization Time or None.



If you select none, the N is red and ‘Data not normalized’ appears in red above the icon.

Hardware and Data Controls

Trap: Enable to turn the ion trap on.

Ion Gauge: Turn on the ion gauge to monitor the trap pressure.

6 Port Valve: Switch the valve position.

Infusion Pump: Start or stop the syringe pump with the flow rate set in Module Attributes.

Purge: Turn on the pump at the Method purge flow rate.

Data Views: Select to view Spectral and Plot screens.

Module Attributes**:** Select to view Instrument Module information.

: Click to display Spectrum and RIC.

: Click to display Optimization Plot.

Default: Click to display Spectrum and RIC.

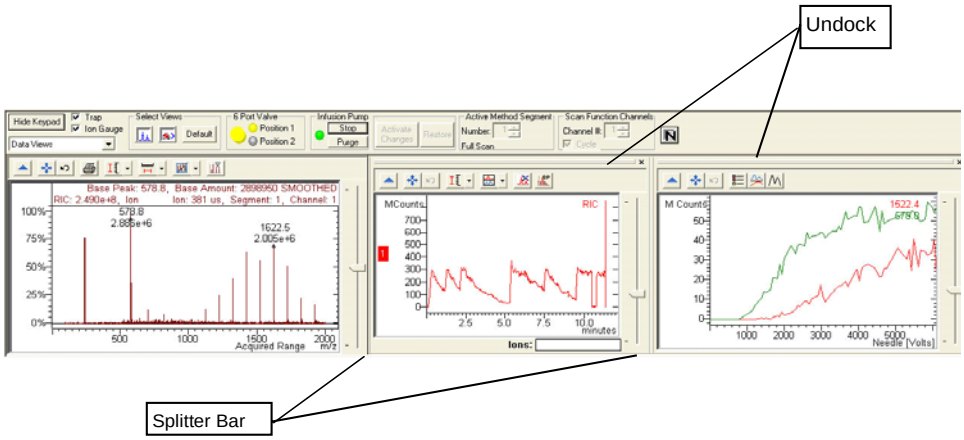
Chromatogram and Optimization Plot Screens

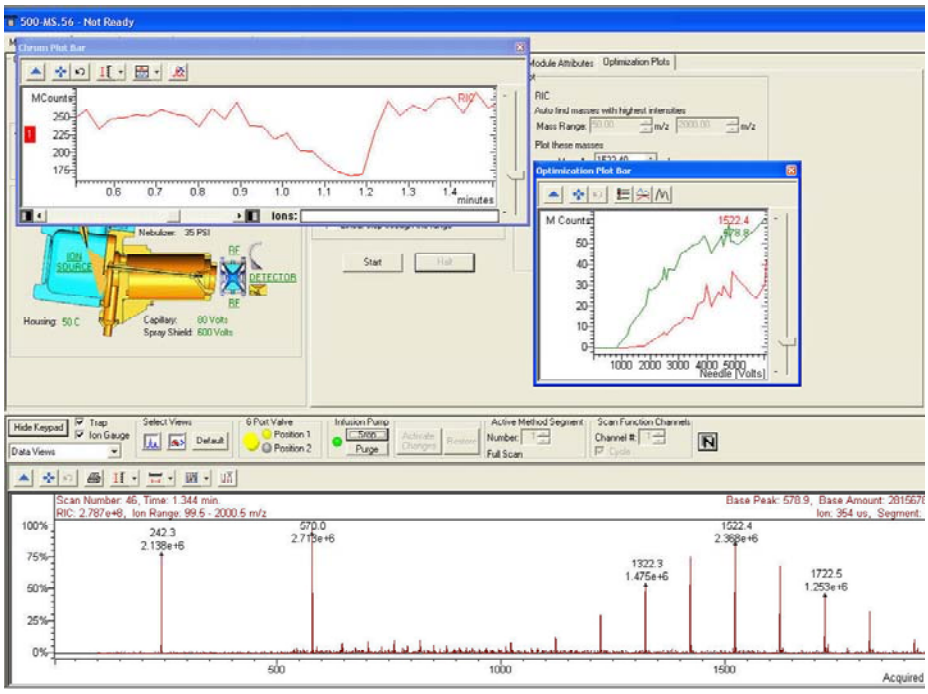
Check either screen to open the plot screens adjacent to the real-time spectrum plot screen.

The Chromatogram plot screen continuously plots the RIC from the real-time spectrum plot screen.

The Optimization Plot screen shows special optimization routines for specified method parameters.

These plots are generated from the Optimization Plots tab and are discussed in the Optimization Plots Tab section. They can be undocked, moved, and resized.





To restore the plot windows to their default location:

- Double-click the title bar of the window.
- Drag the window to the spectrum area.

To reopen a closed view, click the chromatogram button in Select Views to restore it.

Manual Control Tabs

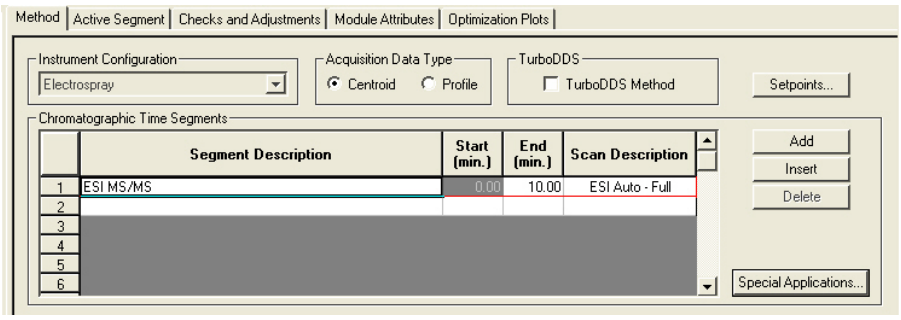
There are five tabs on the 500-MS Keypad.

NOTE: In Manual Control, after making a change in the Method tab view, you must click **Activate Changes** to apply those changes to the active method.

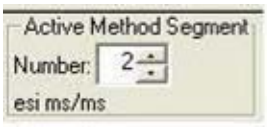
Method and Active Segment Tab

Edit methods created using Method Builder in Manual Control. You can determine the effect of the changes on the mass spectra. Save these changes to the original method or to a new method.

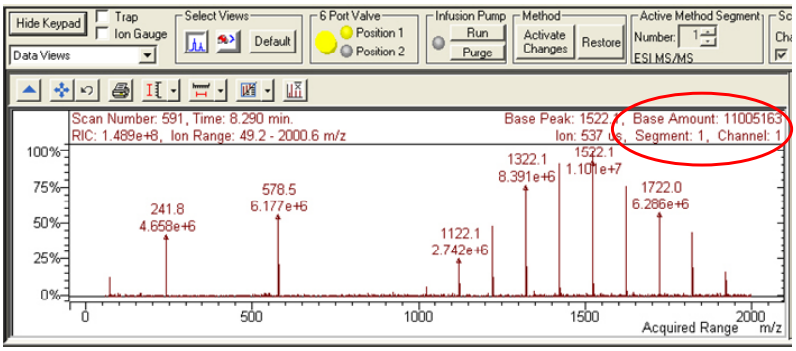
Refer to ‘Building 500-MS Methods’ for more information on method parameters. The set of tab depends on the ionization type (ESI, APCI) and the scan type (Full, MS/MS, etc.).



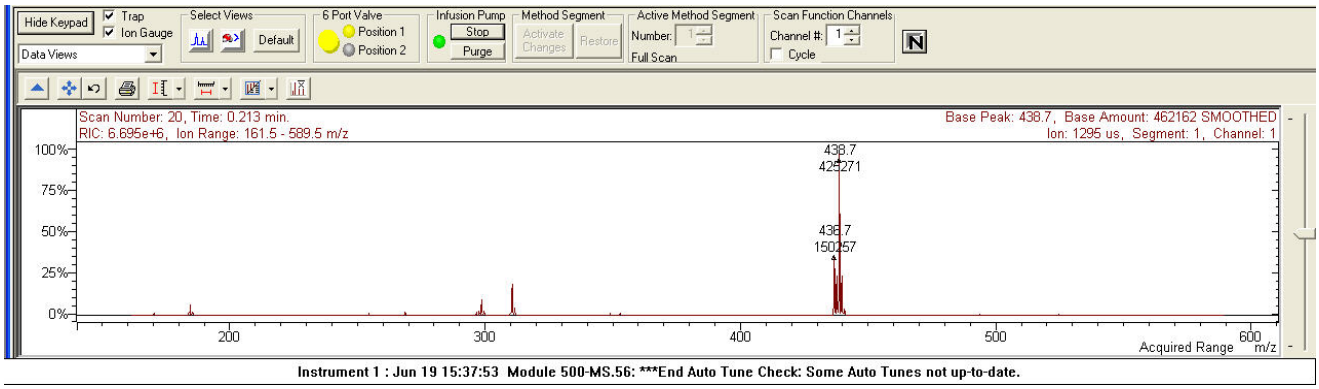
In a multi-segment method, select the segment to edit using the Active Method Segment:



In this multi-segment example, the Active Method Segment is 1 and the Scan Function Channel is 1. Notice that the spectrum has this information in the upper right corner.



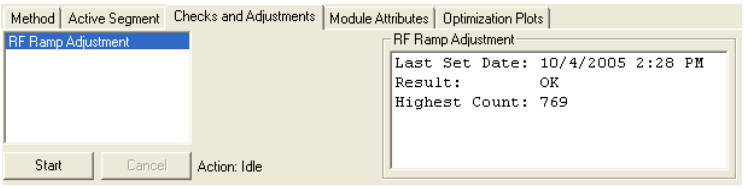
In this multiple reaction monitoring (MRM) example, segment 1 is the active segment. The MRM of two ions creates two channels of spectral data. Use the Scan Function Channel to select the spectrum of channel 1. Notice that the upper right corner of the spectrum has this information. Click cycle to alternate the display between the channels.



In this example, the excitation storage level is increased for channel 1 to eliminate the precursor ion. After making the change, click **Activate Changes** to see the result. Refer to 'Building 500-MS Methods' for more information.

Checks and Adjustments Tab

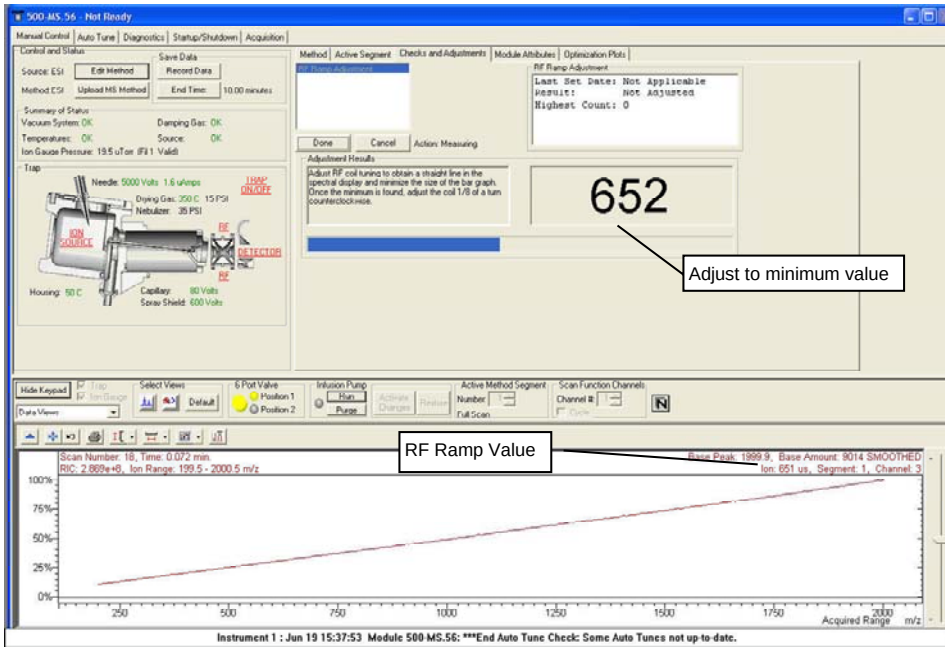
RF Ramp is available in the **Checks and Adjustments** view.



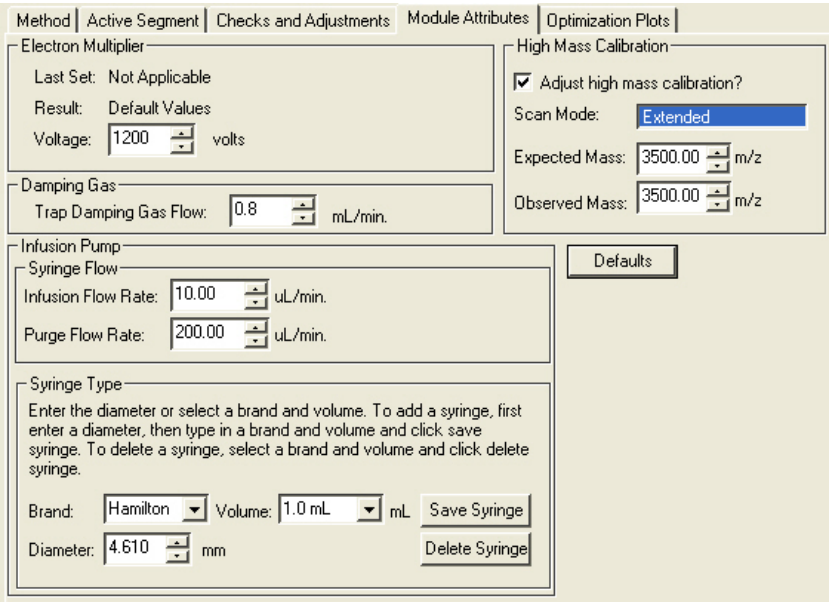
RF Ramp Adjustment

The **RF Ramp Adjustment** determines if the RF voltage is in tune over the entire mass range of the 500-MS. To begin, click **Start**, and then click **Done** when the adjustment is completed.

Open the door to access the RF Adjust on the front panel. After starting the routine, use a screwdriver to adjust the RF adjust screw. Follow the instructions in the Adjustment Results field until the value of the highest part of the graph is at a minimum.



Module Attributes Tab


Method
Active Segment
Checks and Adjustments
Module Attributes
Optimization Plots
Electron Multiplier
Last Set: Not Applicable
Result: Default Values
Voltage: 1200 volts
High Mass Calibration
Adjust high mass calibration?
Scan Mode: Extended
Expected Mass: 3500.00 m/z
Observed Mass: 3500.00 m/z
Damping Gas
Trap Damping Gas Flow: 0.8 mL/min.
Infusion Pump
Syringe Flow
Infusion Flow Rate: 10.00 uL/min.
Purge Flow Rate: 200.00 uL/min.
Defaults
Syringe Type
Enter the diameter or select a brand and volume. To add a syringe, first enter a diameter, then type in a brand and volume and click save syringe. To delete a syringe, select a brand and volume and click delete syringe.
Brand: Hamilton
Volume: 1.0 mL
mL
Save Syringe
Diameter: 4.610 mm
Delete Syringe

Electron Multiplier

You can override the Electron Multiplier voltage determined in Auto Tune.

NOTE: The Electron Multiplier voltage set by Autotune determines the voltage needed for multiplier gain of approximately 10^5. Although this value can be overridden, changing this voltage setting affects the optimal performance of the ion trap. To adjust the multiplier voltage on a single chromatographic time segment, enter the value in the **General Parameters** tab of the Active Segment.

Damping Gas

Set the Damping Gas flow.

NOTE: The helium damping gas maintains mass spectral resolution and improves the trapping of ions. The 500-MS was optimized for a flow rate of 0.8 mL/min. Although this value can be overridden, changing this flow rate setting affects the optimal trapping efficiency of the ion trap. It can also cause the instrument to lose mass calibration.

Infusion Pump

Set the purge and infusion flow rates for the syringe pump. (Alternatively, the infusion flow rate may be specified in Special Applications.)

The infusion pump motor speed is determined from the syringe volume and diameter. There are two preset brands of syringes (Hamilton and SGE). These syringes have different volumes and diameters. To add a custom syringe, follow the directions listed under the Syringe Type header.

After making changes, click **Activate Changes** to apply them.

High Mass Calibration

NOTE: Available only if you have purchased the TurboDDS™ option.

Adding a High Mass Calibration Point

To improve the accuracy of mass assignments, add a mass calibration point greater that the highest values in the Autotune table (2622).

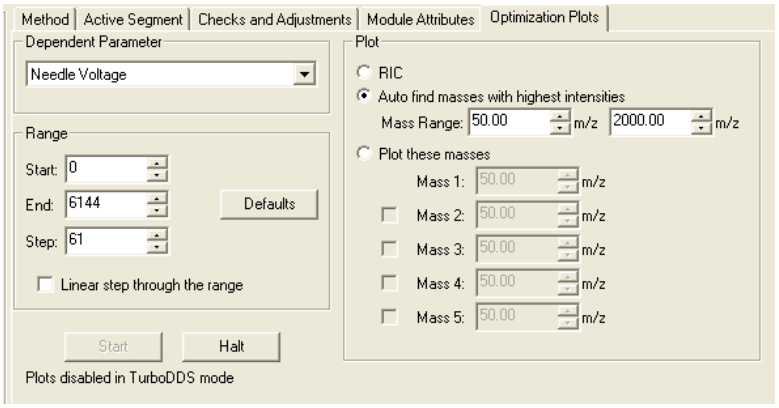
1. Infuse or inject a compound between 2622 and 3500 m/z. Note the observed m/z.
2. Select Adjust High Mass Calibration; enter the observed m/z and the expected exact m/z.
3. Click Activate Changes and the new calibration point becomes part of the mass calibration table.

Do a high mass calibration each time you do an AutoTune. If you leave the **Module Attributes** tab, and then return, Adjust High Mass Calibration will unavailable, but the expected mass value will contain the one you entered.

Optimization Plots Tab

NOTE: Not available in the TurboDDS™ option.

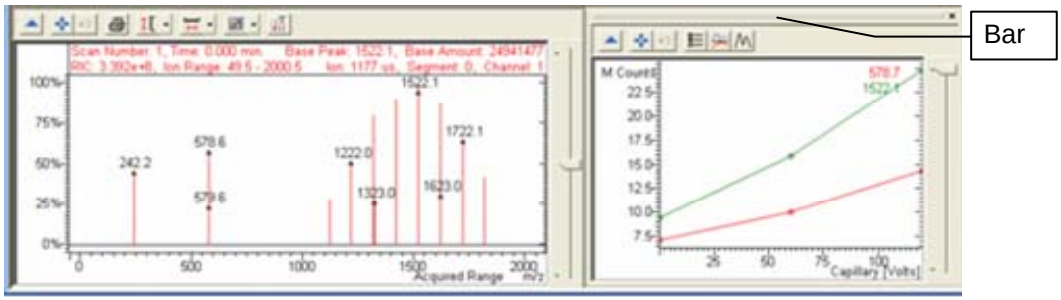
Do MS/MS method development with the built-in syringe pump using the Optimization Plots tab view. Select a dependent parameter, such as Capillary Voltage, and then vary the parameter setting while monitoring and plotting the effect on one or more masses.



NOTE: Before starting, be sure the trap is on, the drying gas temperature is stabilized and the syringe pump is running and has a stable flow.

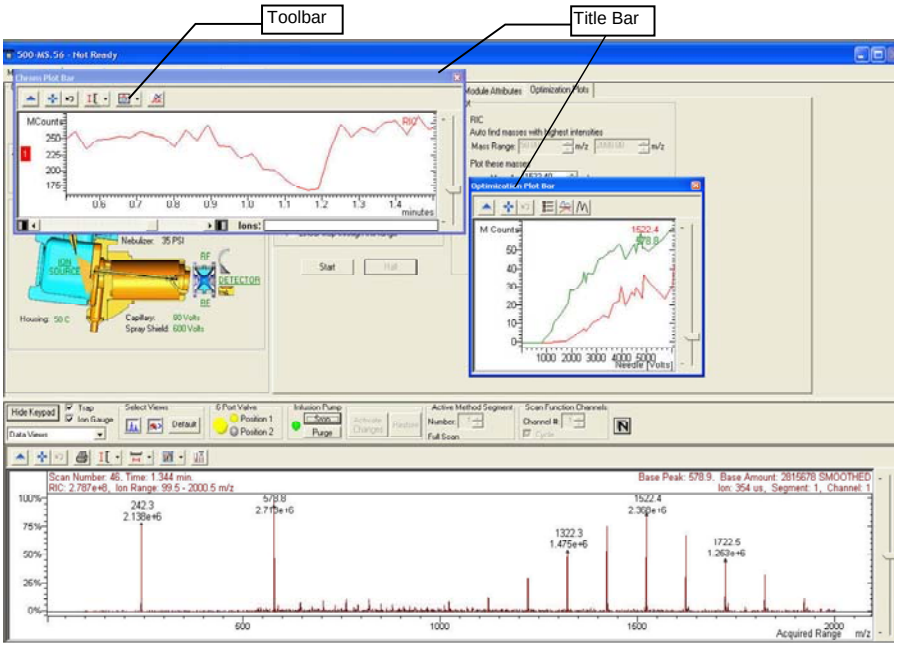
In this example, in order to optimize the Capillary Voltage of two MS/MS precursor ions, the voltage varies from 0**:** 300 in 3 volt steps while plotting the effect on the two precursor ions. The active method must be full-scan to monitor both ions simultaneously.

After clicking **Start**, the Optimization Plot screen automatically appears adjacent to the Spectrum Plot screen. You can click and drag the **Bar** to undock the Plot, move, and resize it.

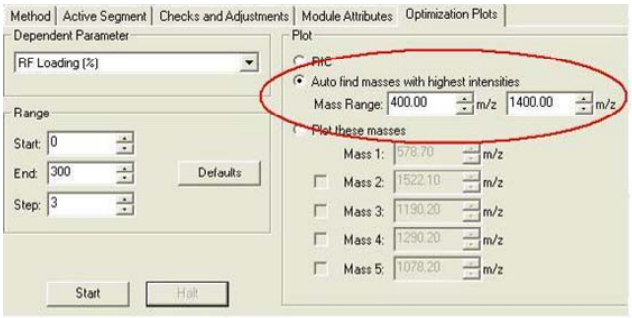


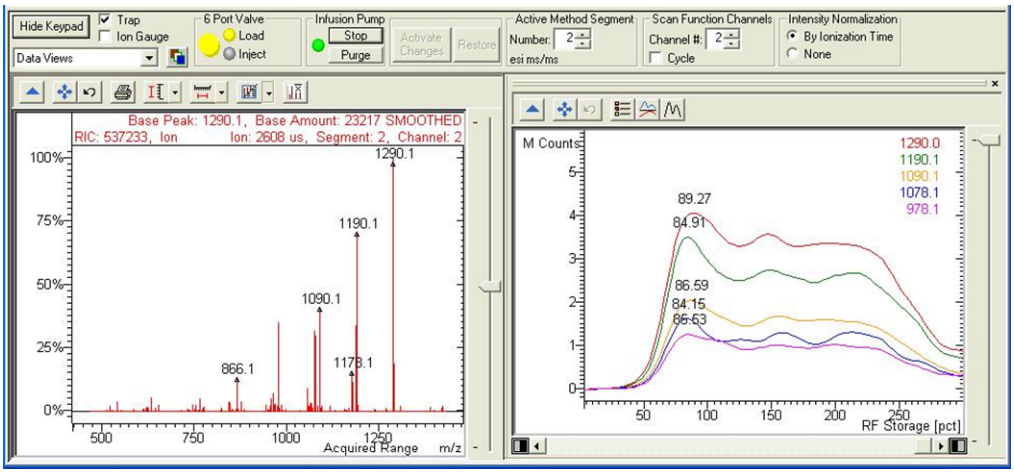
Click the **Toolbar** to overlay, stack, or normalize the plots.

Double-click the **Title Bar** to dock the window to its default location.

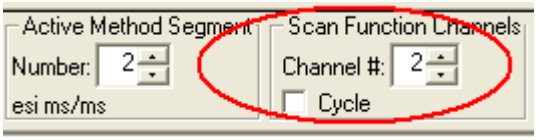


In this example, the RF Loading is varied on a MS/MS segment. Automatically the five largest product masses of the precursor ion (1422.0 *m/z*) are found and the RF Loading effect on these masses is plotted.





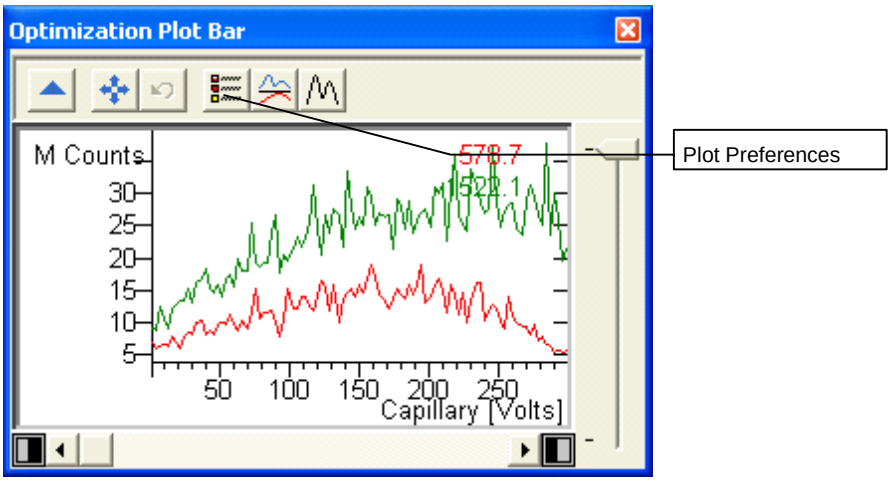
If the active MS/MS method segment is to monitor multiple precursor ions (MRM), the scan function channel must be set to the precursor ion. The **Cycle** checkbox must be unchecked.

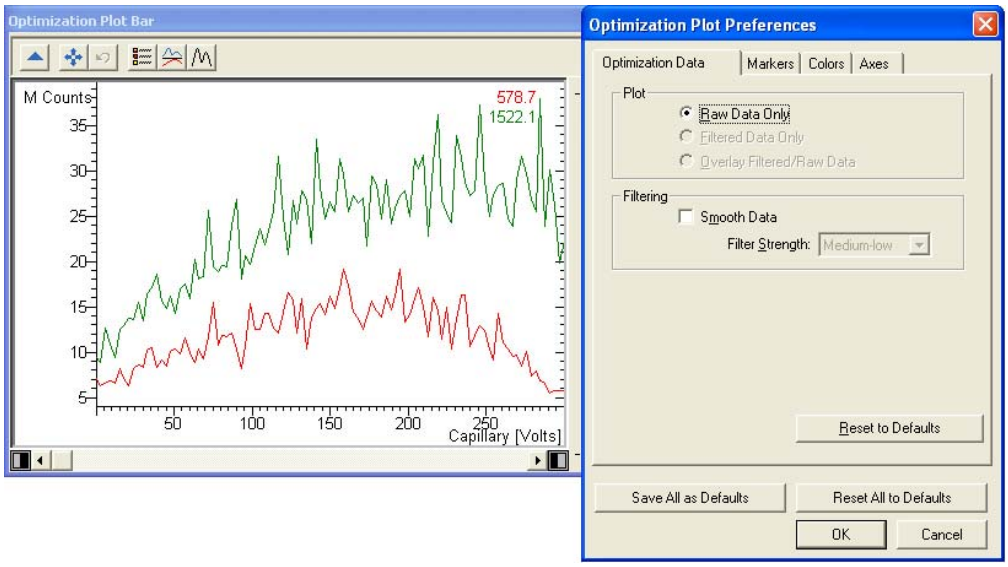


Also, if the active method segment is an MS^n type, the only masses that are plotted for optimization are those produced by the last precursor ion in the list.

Optimization Plot Preferences

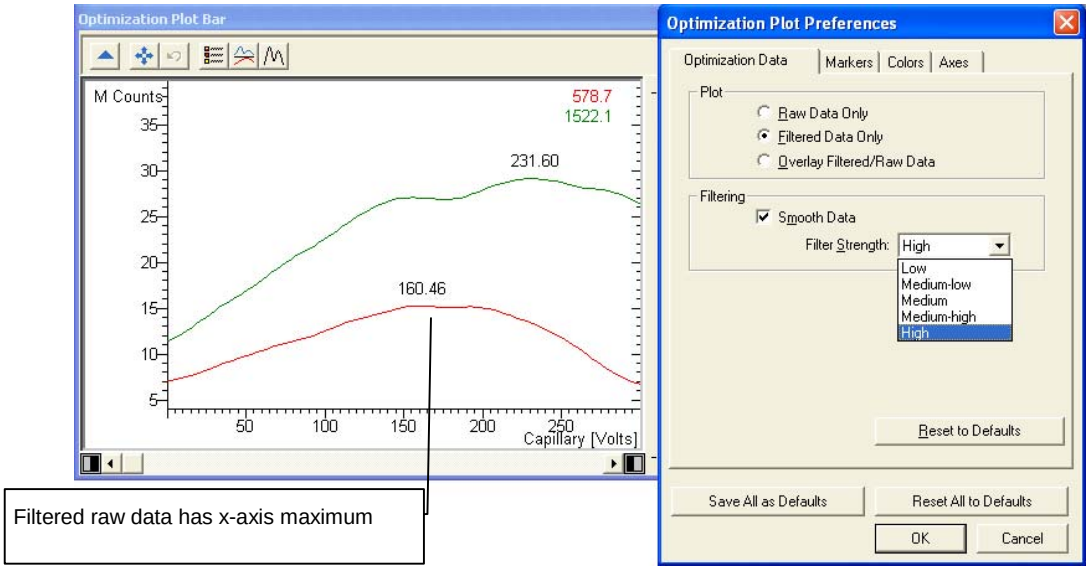
You can customize the optimization plot to select what type of data to display and how it is appears. Click the **Plot Preferences** icon on the plot toolbar.



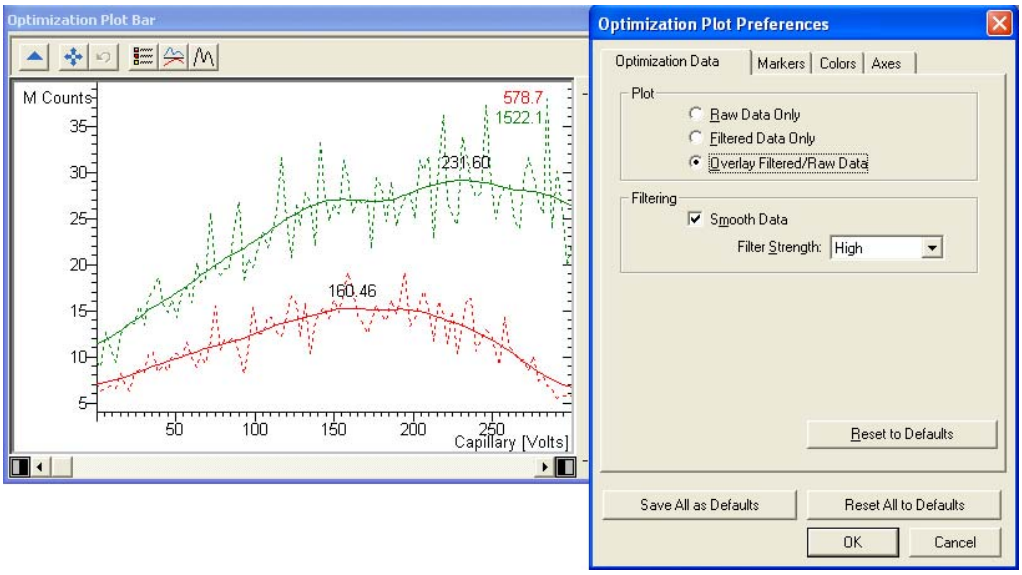


This example displays raw data only. Filter the raw data by checking **Smooth Data** and selecting a **Filter Strength**.

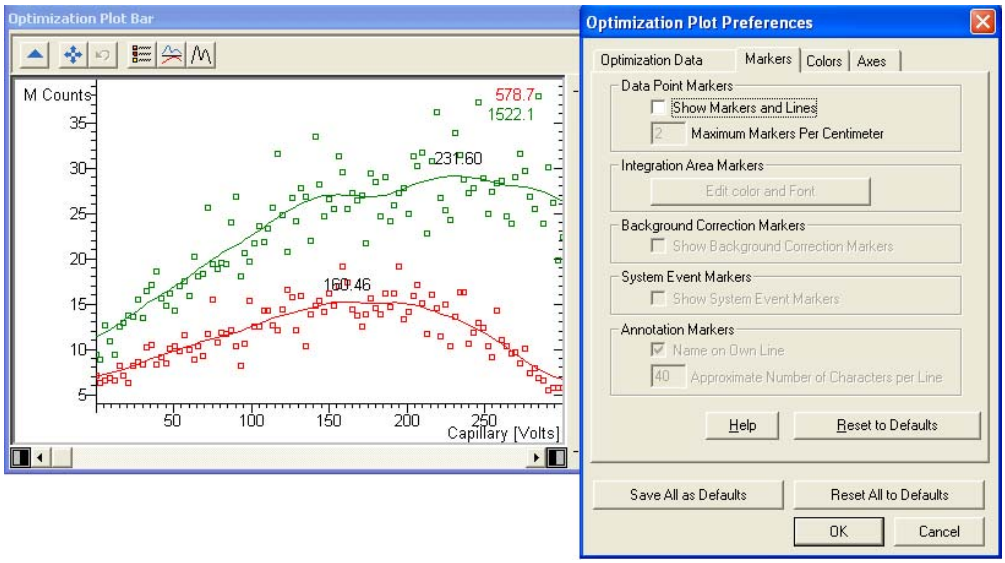
NOTE: The maximum value (Needle Voltage, Capillary Voltage, CID Excitation Voltage, or RF Loading %) is only displayed when the Optimization Plot data is smoothed.



Overlay raw data with the filtered plot.



Use the **Markers** tab to display the individual data point.



Auto Tune Mode

Auto Tune automatically optimizes the 500-MS performance over the entire mass range. Perform Auto Tune when the instrument is first set up and after doing maintenance**.** Before doing Auto Tune, perform the **RF Ramp Adjustment** under the **Checks and Adjustments** tab in Manual Control mode.

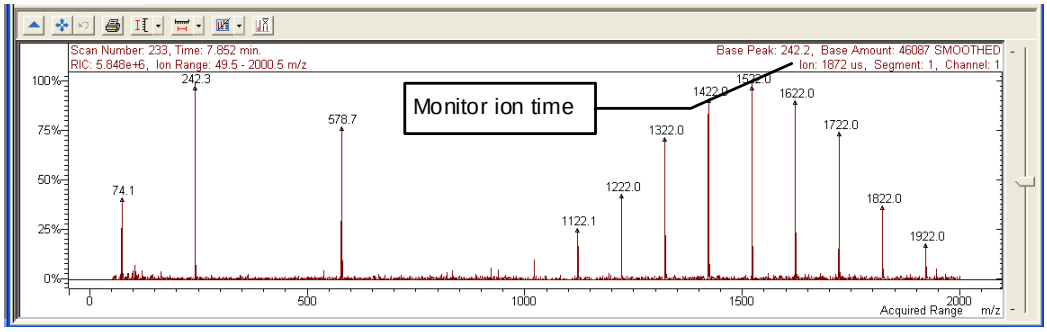
Tune the 500-MS in ESI mode and use these tune settings in APCI mode. You adjust 500-MS parameters (voltages, gain values, etc.) to optimize the signal while infusing the tune solution.

NOTE: Tuning Solution for 500-MS, part number 0393210201. Tuning Solution for TurboDDS™ and Extended Mass range, part number 0393211901.

The Tuning Solution for non-TurboDDS and non-Extended Mass Range work contains the following singly-charged ions:

- Tetramethyl Ammonium Bromide: *m/z* 74.1
- Tetrabutyl Ammonium Bromide: *m/z* 242.3
- Tetradecyl Ammonium Bromide: *m/z* 578.7
- Ultramark: *m/z* 1322.0, *m/z* 1922.0 (also provides ion with these m/z values: 1022.0, 1122.0, 1222.0, 1422.0, 1522.0, 1622.0, 1722.0, 1822.0)

The following is a typical spectrum of this tune solution:



Before doing an Auto Tune, the 500-MS must have a stable infusion flow, the trap should be on, and the drying gas should be stabilized at its set point. Use the following procedure:

1. Ensure that the infusion pump syringe has at least 200 µL of tuning solution. In **Manual Control** mode, activate the method, *500-MS ESI CALIBRATION METHOD.mth*, in the ***VarianWS/500-MS Methods*** directory.
   a. From the **Module Attributes** tab, set the **Infusion Flow Rate** to 10 µL/min.
   b. Turn on the trap and allow the drying gas temperature to stabilize.
   c. Start the infusion pump by pressing **Run** or by pressing the front panel button. (If you have just loaded the syringe, you can click the **Purge** button for a few seconds to move the solution to the needle.)

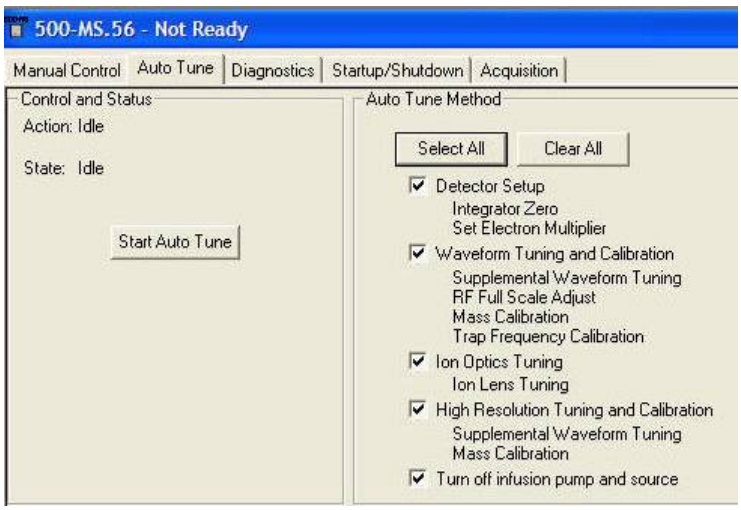
d. Monitor the real-time spectrum. As the tune solution infuses into the trap, the ionization time and ion amplitude (if enabled) readback values fluctuate. The spectrum should look like the previous one (some of the *m/z* values might be shifted). Wait for the ionization to stabilize (ionization time varies by less than 15%).

2. Leave the trap on and the infusion pump running and go to the Auto Tune tab.

After major maintenance is performed (such as cleaning the ion trap or changing the multiplier) or a change to the Trap Damping Gas Flow is made, run all Auto Tune routines. Specific routines may be run periodically as part of a system check.

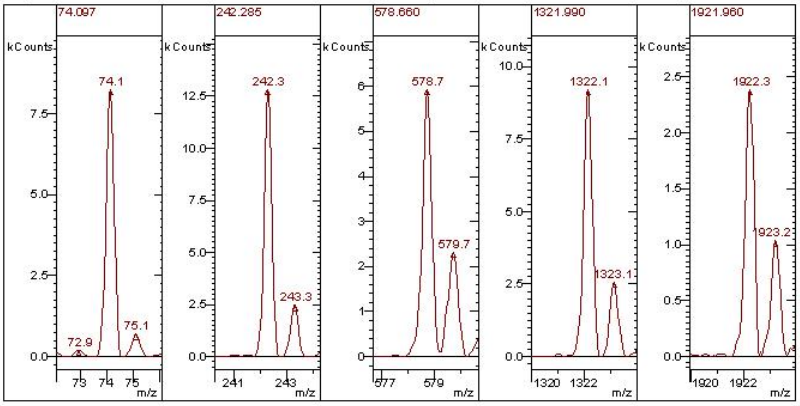
The Auto Tune window has three major sections: the Control and Status display, and the Auto Tune Method display and the Spectrum and Event Message windows. The Auto Tune Method and Control and Status sections are displayed

NOTE: The High Resolution option is only available if you have purchased the TurboDDS option.



AutoTune reports can be printed. The following example is of a TurboDDS™ AutoTune report

Varian 500-MS Autotune Summary Report - 4/2/2007 3:06 PM



Tune Parameters

Multiplier Voltage	1600 Volts	OK
Integrator Zero Value	409 DAC value	OK
RF Full Scale Adjust	4103 DAC values	OK
Trap Frequency Calibration	225.9/ 225.6/ 79.7	OK
Lens Tune Positive	-15.3/ -48.2/ -18.3/ -24.7	OK
Lens Tune Negative	15.0/ 29.3/ 15.6/ 34.4	OK

Calibration Parameters

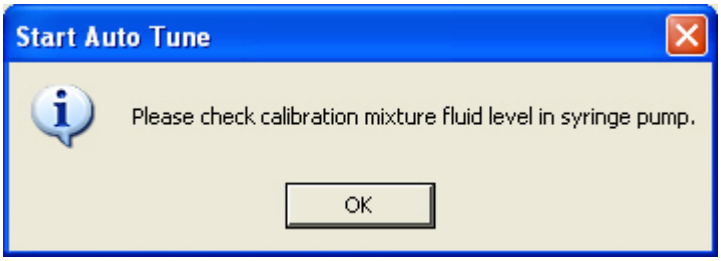
Mass Calibration Enhanced Mode	OK
Mass Calibration Standard Mode	OK
Mass Calibration Extended Mode	OK
Mass Calibration HiRes Mode	OK
Supplemental Waveform Enhanced Mode	OK
Supplemental Waveform Standard Mode	OK
Supplemental Waveform Extended Mode	OK
Supplemental Waveform HiRes Mode	OK

Instrument Parameters

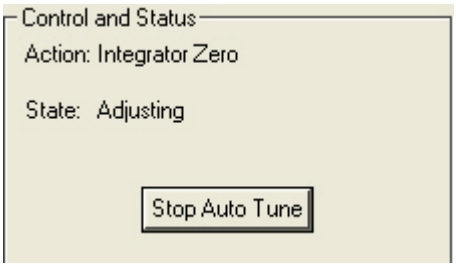
Foreline Pressure	835 mTorr	OK
Forward Turbo Power	52 Watts	OK
Rear Turbo Power	30 Watts	OK
Foreline Pump 1 Power	397 Watts	OK
Foreline Pump 2 Power	392 Watts	OK

Control and Status

Start Auto Tune by clicking **Start Auto Tune**. Click **Stop Auto Tune** to stop Auto Tune. The Start Auto Tune window opens reminding you to check the syringe pump. Click **Ok**.

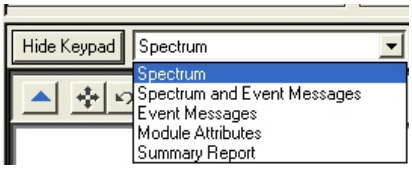


In the Control and Status section, the Action field displays the tuning routine in progress and the State field displays whether the 500-MS is currently executing an auto tune routine or is idle.

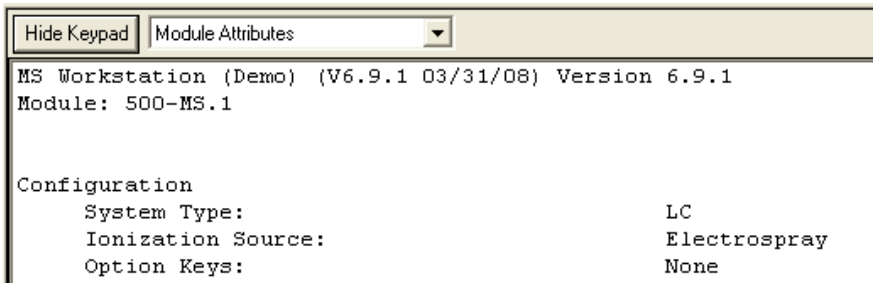


Spectrum and Event Message Window

The following shows the left side of the auto tune screen. You select information to display during the auto tune tests. **Hide Keypad** causes the upper portion of the screen to disappear and the lower portion to expand.



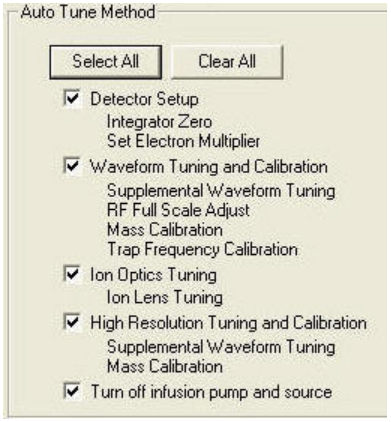
The Module Attributes displays the information stored from the last auto tune. When you are finished, performing the auto tune you can view this information, which is stored in an auto tune log.



Auto Tune Methods

The auto tune routines automatically set up, tune, and calibrate the mass spectrometer. In addition, you can select to turn off the infusion pump and source after tuning completes.

Auto Tune routines are generally performed in the order listed, beginning with Detector Setup and ending with Ion Optics Tuning. All of the auto tune methods are selected if you click **Select All.** They are cleared if you click **Clear All**.

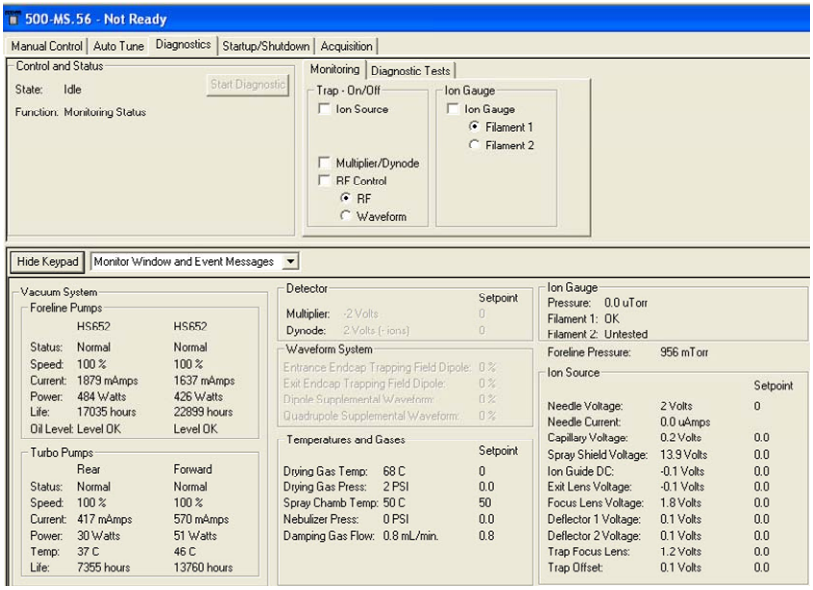


To perform Auto Tune click the check boxes, and click **Start Auto Tune**. Each of the routines you selected will be executed. Stop the auto tune by clicking **Stop Auto Tune**.

NOTE: Run all available routines during an Autotune. However, any of the three specific routines may be run periodically as part of a system check.

Diagnostics Mode

In diagnostics mode monitor the status of the vacuum system, applied voltages, gases and temperatures in real time. It also offers diagnostic tests. The Diagnostics Window is divided into two panes, the Keypad above and the Module Window and Event Messages display below.



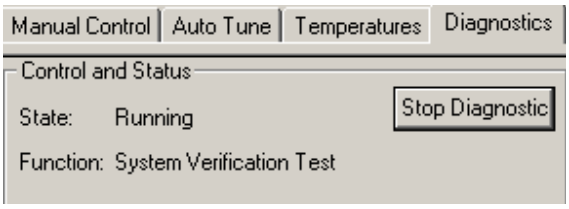
Keypad Field

The upper portion of the screen is divided into three sections: **Control and Status**, **Monitoring**, and **Diagnostics Tests**.

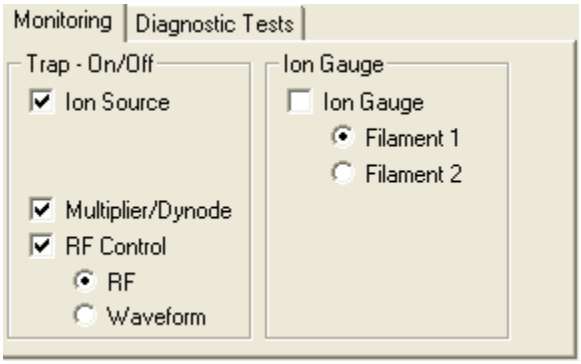
Control and Status

The State field indicates ***Idle*** when diagnostic tests are not running or ***Running*** if tests are in progress. The Function field indicates ***Monitoring Status*** unless a diagnostic test is running, in which case the name of the current test is displayed. **Start Diagnostic** is grayed out unless a test is selected in the **Diagnostic Tests** tab dialog.

After you, select a diagnostic test and click **Start Diagnostic**, the test runs and the results are displayed in the Event Messages at the bottom of the screen. The **Start Diagnostic** button becomes **Stop Diagnostic**.



Monitoring Tab Dialog



The Trap On/Off section is where you select the ion trap features to monitor. If you select Ion Source, the Ion Source section of the Monitor window becomes active. If you select Multiplier/Dynode, the voltages of the electron multiplier and conversion dynode are displayed in the Trap field of the Monitor window. If you select RF Control with RF selected, you see the Entrance and Exit Trapping Field Dipole values. If the Waveform radio button is selected with RF Control, the Dipole and Quadrupole Supplemental Waveform values are displayed.

If the Ion Gauge option is selected, the Ion Gauge pressure in µtorr is displayed in the Ion Gauge field of the Monitor window.

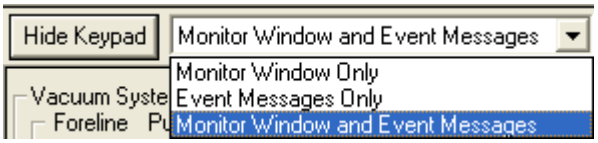
Diagnostic Tests Tab Dialog

The **Quick Verify** test checks the entire system to see if the 500-MS hardware is operating properly. Results of the test are displayed in the event messages window at the bottom of the screen. If a failure is reported, go to the relevant section of the Troubleshooting Guide in the 500-MS Hardware Operation Manual.

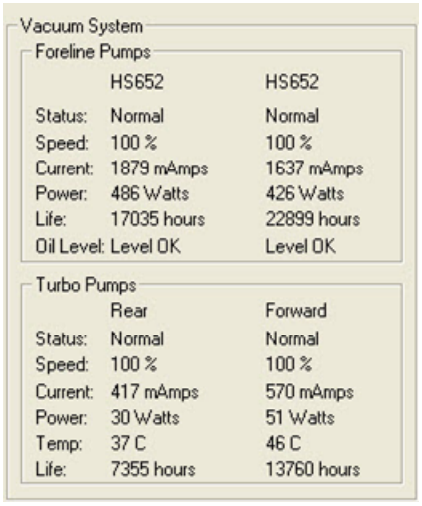
Configuring the Monitor and Event Messages Display

You can display the Monitor window and Event Messages in a larger area by clicking **Hide Keypad** or recover the Keypad by clicking **Show Keypad**.

From the menu, you can select to display the Monitor Window or Event Messages alone or in combination.

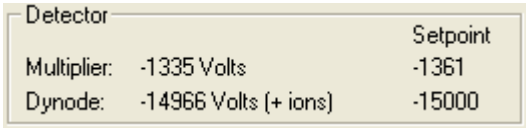


Vacuum System



The foreline pump and turbomolecular pump current and power readings during routine operation are shown. You can check the oil level in the foreline pumps. The running lifetime (in hours) of both turbo pumps and foreline pumps is displayed. When a pump is replaced, the lifetime hours counter is reset.

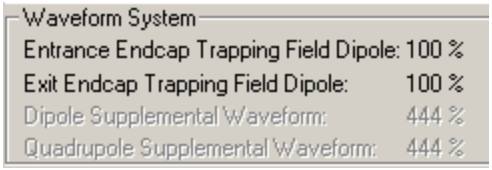
Trap



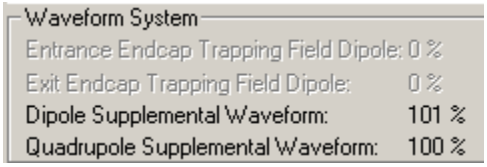
Select **Multiplier/Dynode** in the **Monitoring** tab view to display the readings for the multiplier and conversion dynode voltages. The multiplier reading is negative and reflects the voltage drop between ground and the entrance of the multiplier.

The dynode voltage should be close to 15,000V; the polarity is the opposite of the charge of ions being detected.

Waveform System

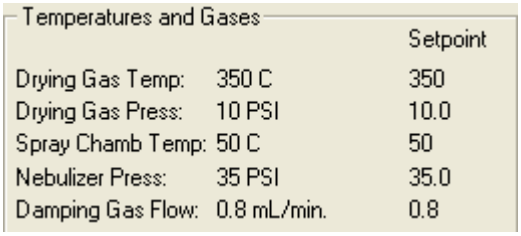

Waveform System
Entrance Endcap Trapping Field Dipole: 100 %
Exit Endcap Trapping Field Dipole: 100 %
Dipole Supplemental Waveform: 444 %
Quadrupole Supplemental Waveform: 444 %

Select **RF Control** and **RF** button in the **Monitoring** tab view to display the readings for the Entrance/Exit End Cap Trapping Field Dipole. The values should be 100 ± 1%.


Waveform System
Entrance Endcap Trapping Field Dipole: 0 %
Exit Endcap Trapping Field Dipole: 0 %
Dipole Supplemental Waveform: 101 %
Quadrupole Supplemental Waveform: 100 %

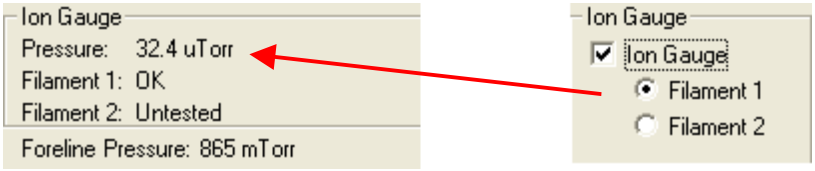
Select **RF Control** and the **Waveform** radio button in the Monitoring tab view to display the Dipole and Quadrupole Supplemental Waveform readings. The values should be near 100%.

Temperatures and Gases


Temperatures and Gases
Setpoint
Drying Gas Temp: 350 C 350
Drying Gas Press: 10 PSI 10.0
Spray Chamb Temp: 50 C 50
Nebulizer Press: 35 PSI 35.0
Damping Gas Flow: 0.8 mL/min. 0.8

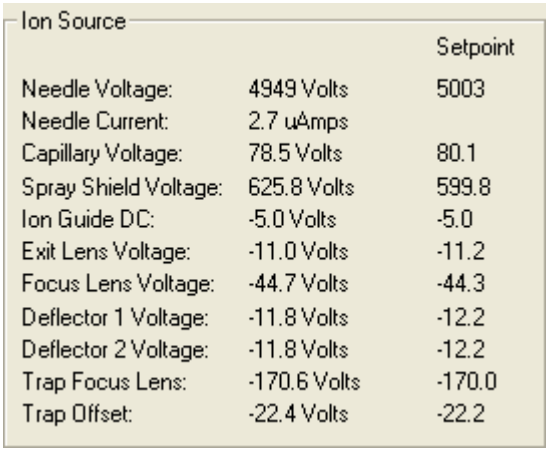
The temperatures of all heated zones are shown with the gas pressures and the damping gas flow. The ion source must be on to view the drying gas and nebulizer readbacks. In APCI mode, the Vaporizer temperature and pressure readbacks are displayed.

Ion Gauge



If the Ion Gauge is selected, the ion gauge pressure in µTorr is displayed in the Ion Gauge field of the Monitor window. Select one of the two filaments to produce the reading. The foreline pump pressure is just below this.

Ion Source (ESI)


Ion Source
Setpoint
Needle Voltage: 4949 Volts 5003
Needle Current: 2.7 uAmps
Capillary Voltage: 78.5 Volts 80.1
Spray Shield Voltage: 625.8 Volts 599.8
Ion Guide DC: -5.0 Volts -5.0
Exit Lens Voltage: -11.0 Volts -11.2
Focus Lens Voltage: -44.7 Volts -44.3
Deflector 1 Voltage: -11.8 Volts -12.2
Deflector 2 Voltage: -11.8 Volts -12.2
Trap Focus Lens: -170.6 Volts -170.0
Trap Offset: -22.4 Volts -22.2

After the ion source is turned on, these readbacks are displayed. The needle voltage, set in the active method, produces a readback. The capillary and spray shield voltages are also set in the active method. The other setpoints are from the Autotune procedure.

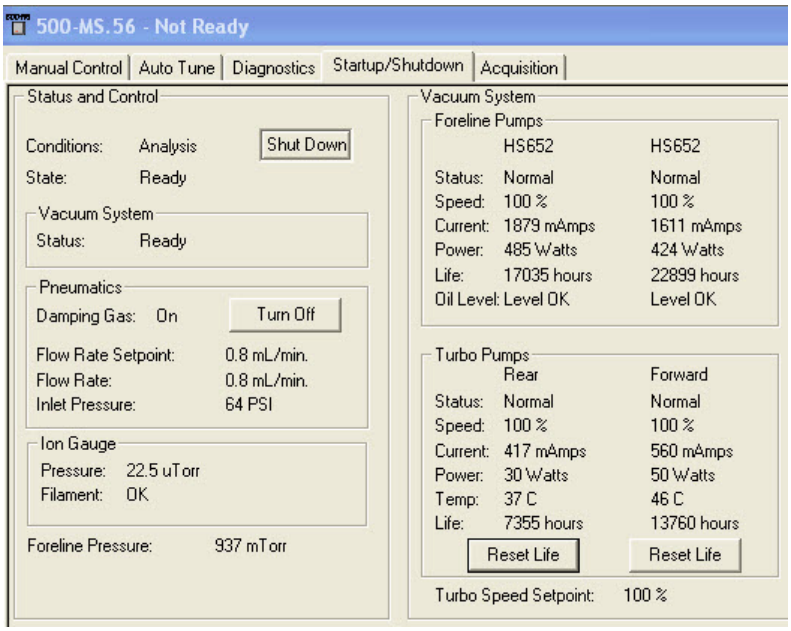
NOTE: In APCI mode, the Needle Voltage and Current readbacks refer to the Corona Needle. In this case, the Corona Needle Current set in the active method produces a Corona Needle Voltage readback.

Event Messages Display

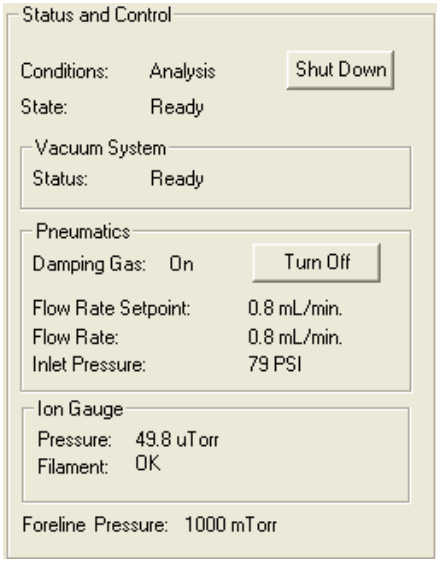
Displays messages when diagnostic tests are performed.

Startup/Shutdown Mode

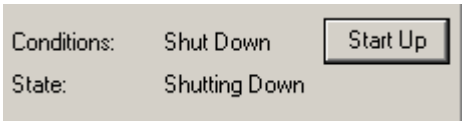
Use the **Startup/Shutdown** tab to properly shutdown the 500-MS for maintenance and to start it up again. Monitor the vacuum system and damping gas pneumatics during Startup and Shutdown in Manual Control.

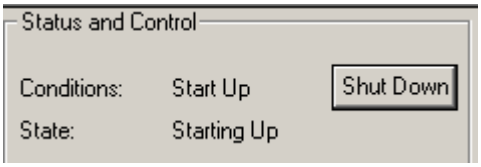


Status and Control



The **Conditions** field displays either Analysis or Shut Down. The **State** displays Ready, Shutting Down, or Starting Up.





Vacuum System

Vacuum system displays either Not Ready or Ready. The speed of the turbomolecular pumps must be 100% for Ready to be displayed.

Pneumatics

Displayed if auxiliary helium damping gas is flowing to the ion trap. Damping gas flow is required for adequate mass spectral resolution and sensitivity. It is set at 0.8 mL/min, which is the optimum flow rate for most applications. This flow rate is set in the **Module Attributes** tab in Manual Control. The damping gas automatically turns off during Shutdown and automatically turns on when Startup is complete. If necessary, the damping gas flow can be turned off for diagnostic purposes. When off, the flow remains off in all modes except for Acquisition mode, where it automatically turns on.

Ion Gauge

Monitor the internal pressure here. The ion gauge filament is automatically turned on when you open the Startup/Shutdown window. It turns off automatically after 20 minutes.

Vacuum System

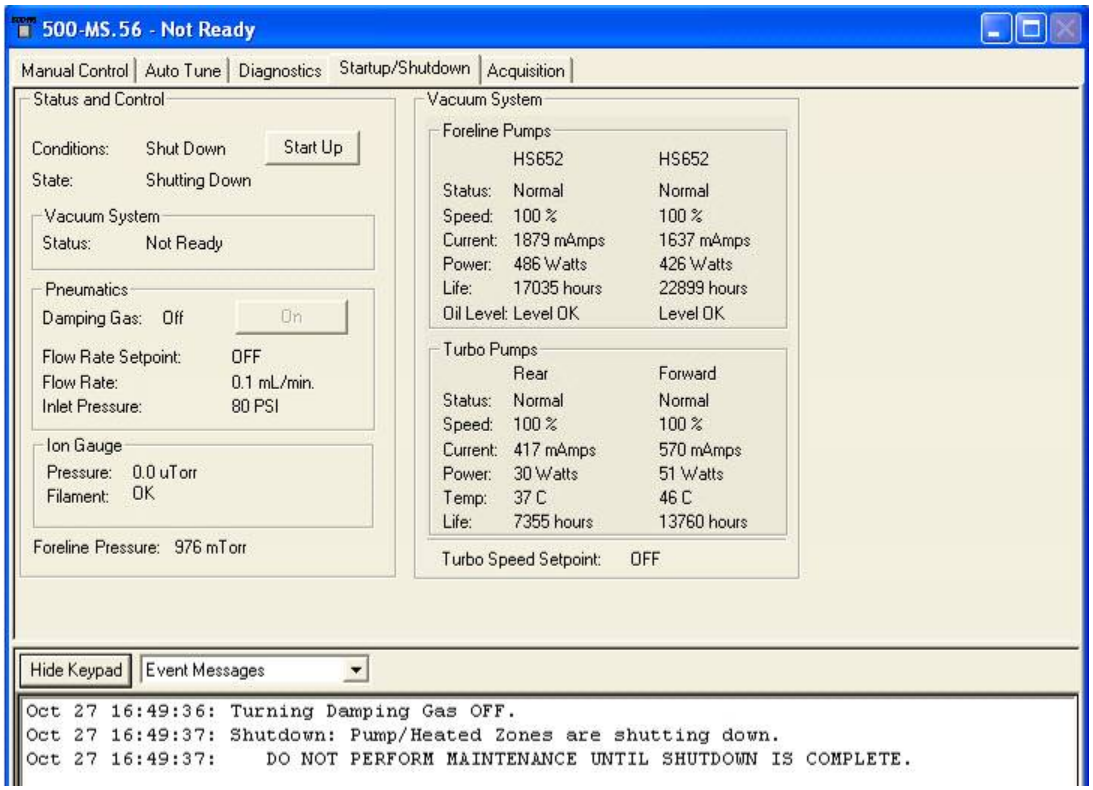
Monitor the status of the foreline pumps and turbomolecular pumps during Shutdown and Startup.

The turbomolecular pumps speed is 100 ± 2% during normal operation. The rate at which the turbomolecular pumps start up is proportional to how long they have been shut down. After short shutdowns, the time to reach 100% speed should be less than 2 minutes. After extended shutdowns, the pump speed increases in stages related to power consumption. This slower startup improves pump lifetime.

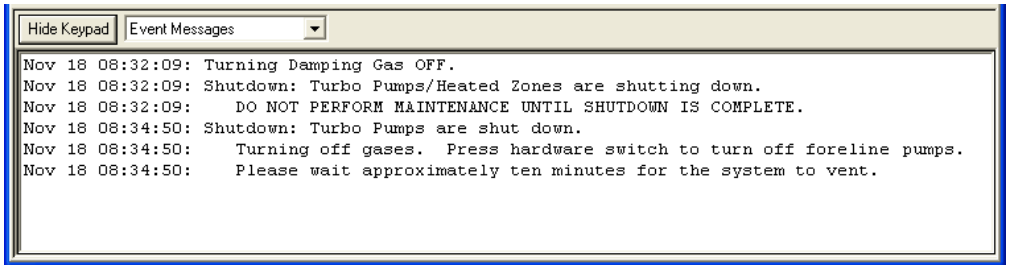
NOTE: Failure to reach 100% pump speed in a reasonable time indicates a vacuum leak and corrective action should be taken. Go to the Troubleshooting section in the **500-MS Hardware Operation Manual**.

Shutting Down the System

To shut down the 500-MS, click **Shut Down** in the upper left corner of the screen. The damping gas and heaters turn off and the speed of the turbo pumps gradually reduces to 35%. Cancel the shutdown procedure by checking **Start Up**.

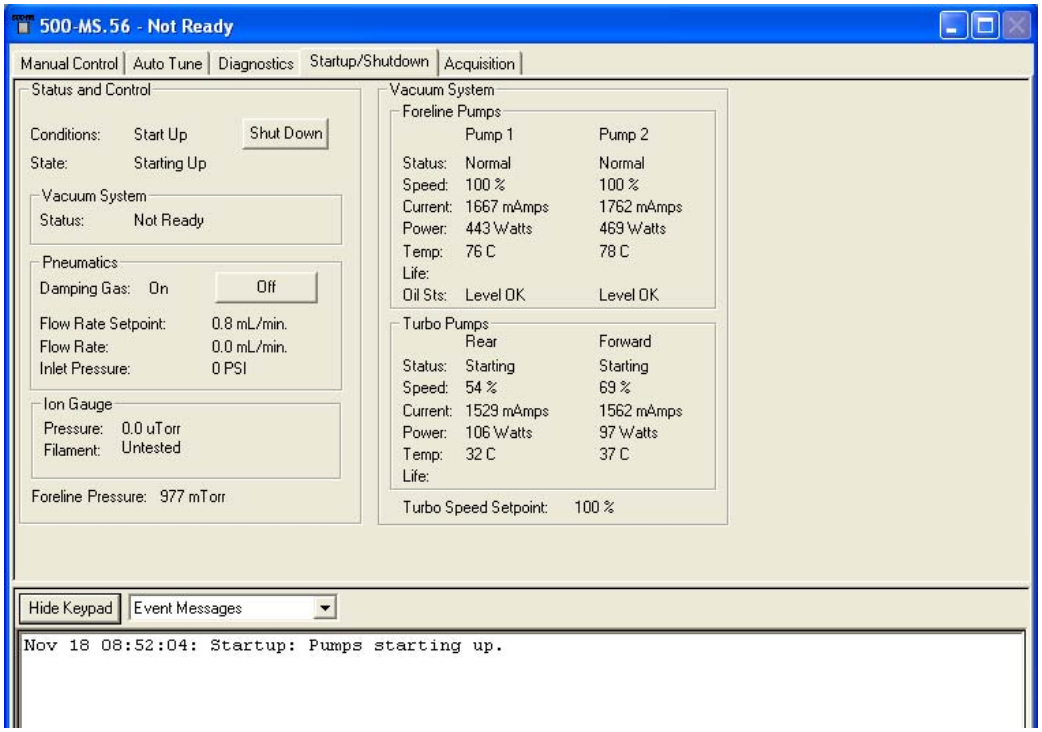


After the turbo pumps reach 35% speed, the remaining gases turn off. Close **System Control** and turn off the foreline pumps by turning off the 500-MS hardware from the front panel switch. The system should be fully vented after 10 minutes.

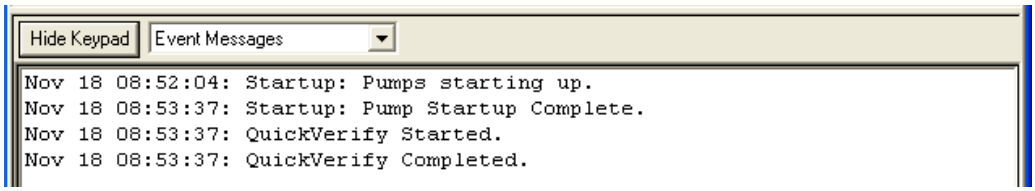

Hide Keypad
Event Messages
Nov 18 08:32:09: Turning Damping Gas OFF.
Nov 18 08:32:09: Shutdown: Turbo Pumps/Heated Zones are shutting down.
Nov 18 08:32:09: DO NOT PERFORM MAINTENANCE UNTIL SHUTDOWN IS COMPLETE.
Nov 18 08:34:50: Shutdown: Turbo Pumps are shut down.
Nov 18 08:34:50: Turning off gases. Press hardware switch to turn off foreline pumps.
Nov 18 08:34:50: Please wait approximately ten minutes for the system to vent.

Starting Up the System

Restart the system by turning on the 500-MS hardware from the front panel switch and then restarting the **System Control**.



During start up, watch Pump Spin Speeds increase. The software locks in Startup/Shutdown and Diagnostics modes until the pump speeds reach 100%. (You can also monitor the startup from the Diagnostics tab). The Damping Gas flow automatically turns on after the Pump Spin Speed reaches 100% and **Quick Verify** runs. **Quick Verify** checks the entire system to determine if everything is operating properly.

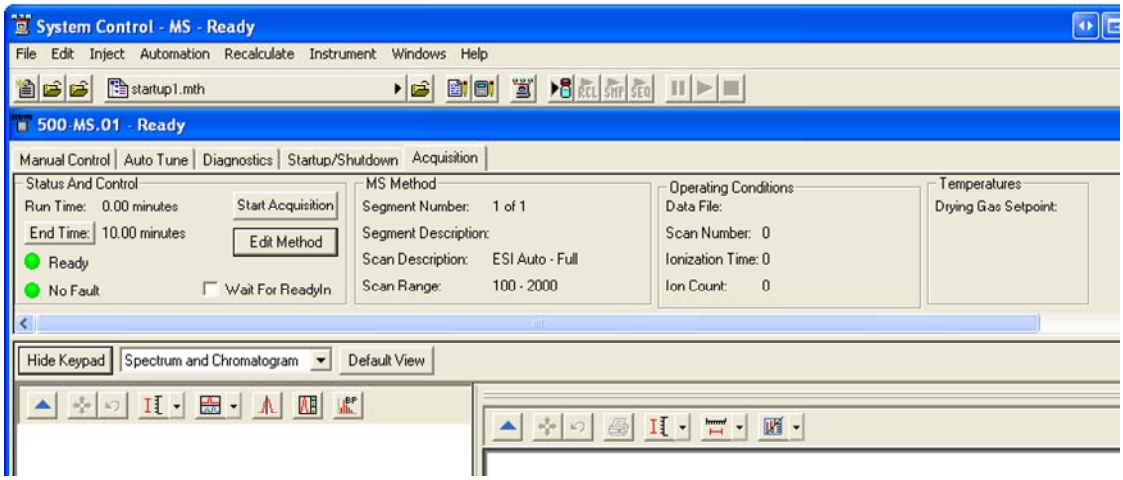

Hide Keypad
Event Messages
Nov 18 08:52:04: Startup: Pumps starting up.
Nov 18 08:53:37: Startup: Pump Startup Complete.
Nov 18 08:53:37: QuickVerify Started.
Nov 18 08:53:37: QuickVerify Completed.

NOTE: If any hardware problems were detected upon startup or from the **Quick Verify** test, the system automatically loads the **Diagnostics** tab where you can determine the problem.

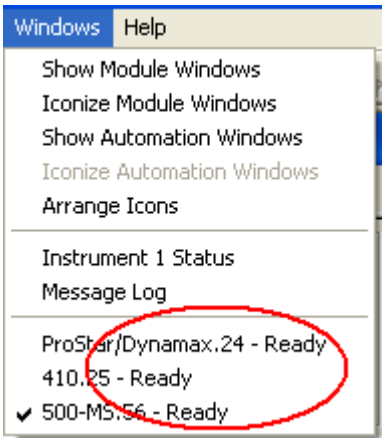
Acquisition Mode

About Running Analyses

Selecting the **Acquisition** tab activates the 500-MS module. If you start an analysis while the instrument is in another mode, the software automatically shifts the MS module into Acquisition mode.



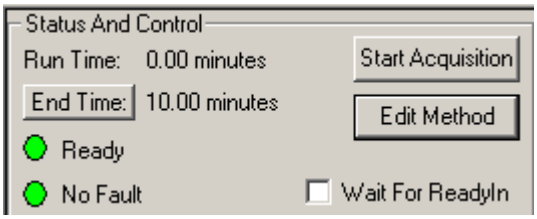
The yellow (Not Ready) light turns green (Ready) to indicate that the 500-MS is ready for analysis. If other LC modules are not ready, Not Ready messages are at the top of the System Control window. After all the hardware modules come to a ready state, the Not Ready messages change to Ready. To determine the ready states of each component, go to the top pull-down menu under Windows and see the states for the modules.



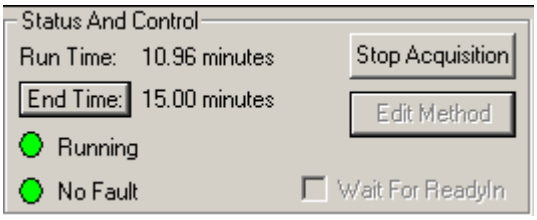
After all components are ready, you can start an analysis.

An analysis can be run as a single sample or through an automated sequence. To run a single sample see "Injecting Single Sample" on page 101. To multiple samples see "Injecting Multiple Samples" on page 105.

Status and Control

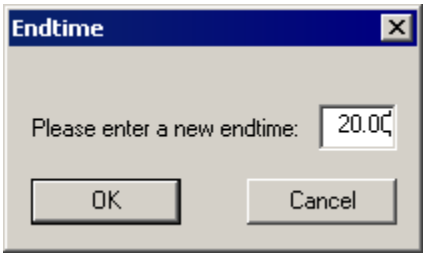


This is how the Status and Control field looks before an acquisition starts. The Run Time is 0.00 minutes because the run has not started. The End Time is the run time specified in the active method. The Ready and No Fault lights are green. You can use the **Start Acquisition** button to override automation and start a run automatically even before the system is Ready. The file name of this type of run is named using the last data file naming convention specified in the last SampleList used in Acquisition mode. Click **Edit Method** to open the Method Builder and modify the method. You are prompted to re-activate the method after you save changes and return to System Control. **Wait For ReadyIn:** This feature is used with external devices that are not networked in to the system such as fraction collectors. If **Wait For ReadyIn** is selected, the system waits for an external Ready signal before starting an acquisition.



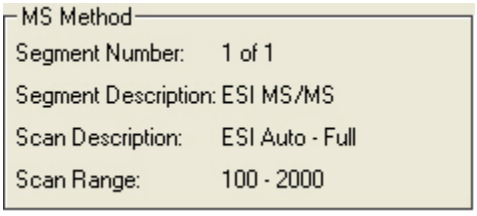
After an acquisition begins, the run time increases in 0.01 minute increments. If you need to increase the end time for the run, click **End Time** and select the

desired time. The **Stop Acquisition** button terminates the MS run but not other LC hardware runs. You stop the runs of those modules individually.

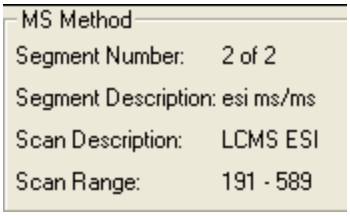


MS Method

Before an acquisition starts, the active method is in Segment 1, which may be a data collection delay segment with the trap turned off for the segment duration.

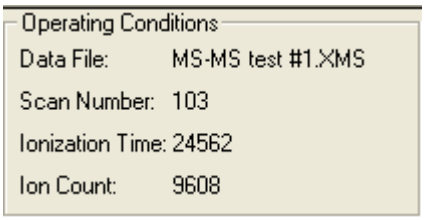


After a run starts, the Segment Number increments after the segment start time is reached. The Segment Description is the user-entered description in the Segment Description field of the MS Acquisition Method in the active method. The Scan Description indicates the ionization and ion preparation methods chosen in the Scan Type/Group and Ionization fields of the Method Segment field of the method. Scan Range is the acquisition mass range.



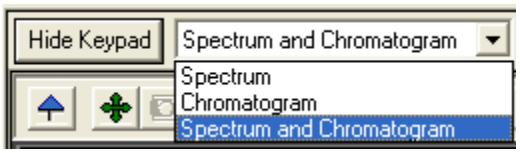
Operating Conditions

Data File shows the name of the file currently being acquired. The Scan Number, Ionization Time and Ion Count for the current scan are displayed in their respective fields. This display updates scan-by-scan throughout the run.



Spectrum and Chromatogram Windows

Below the keypad, select the Spectrum or the Chromatogram plot or both.



Customize the appearance of the Chromatograph and Spectrum View:

- Chromatogram: Select Chromatogram Plot Preferences
- Spectrum: Select Spectrum Plot Preferences

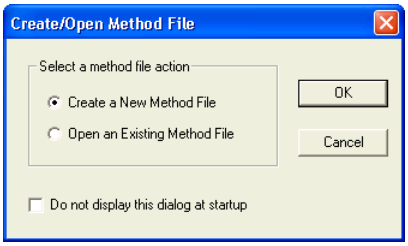
For more information on acquiring data in Acquisition mode, see “Injecting Samples” on page 96.

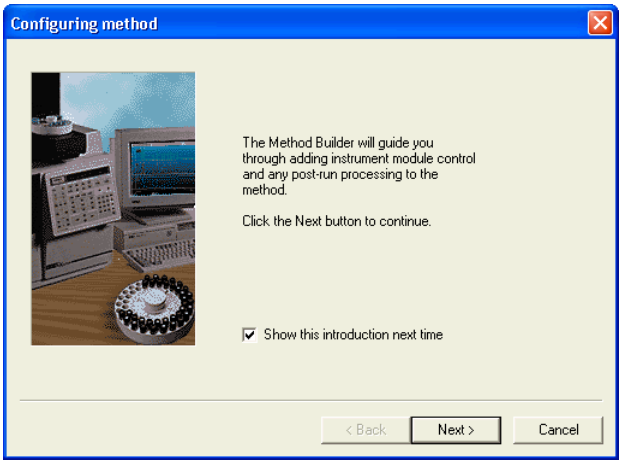
Building 500-MS Methods

Creating a New Method

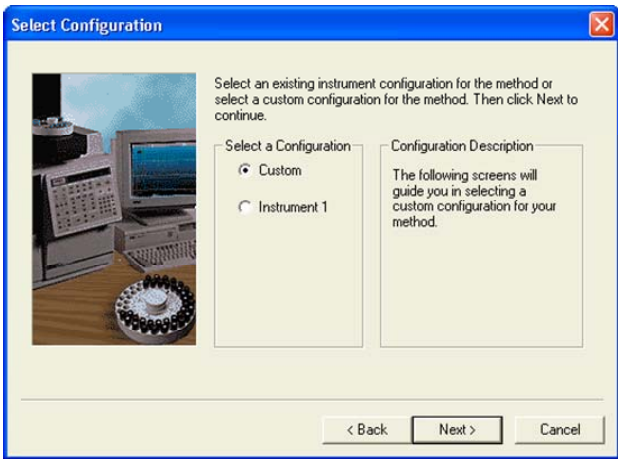
Click **Method Builder** on the **Workstation** Toolbar to open the following.

Click **Create a New Method File** and the Star Assistant Wizard opens.

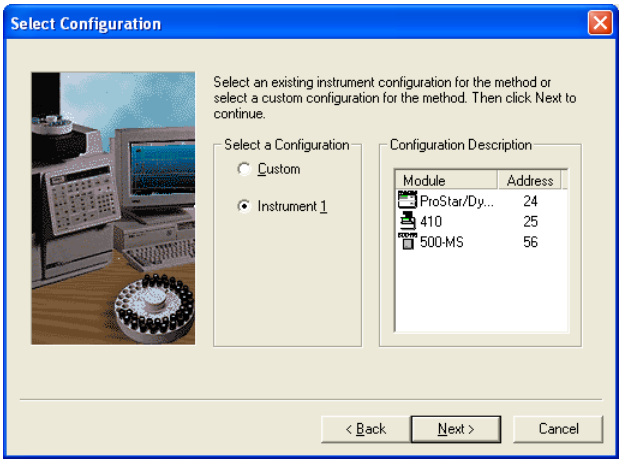



Configuring method
The Method Builder will guide you through adding instrument module control and any post-run processing to the method.
Click the Next button to continue.
Show this introduction next time
< Back
Next >
Cancel

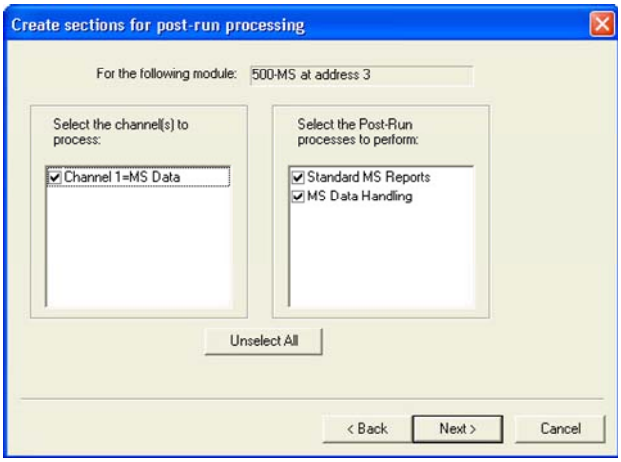
Select the instrument configuration for the method, either Custom or Instrument 1. You can use Custom configuration to create methods on a PC remote from the instrument.


Select Configuration
Select an existing instrument configuration for the method or select a custom configuration for the method. Then click Next to continue.
Select a Configuration
Custom
Instrument 1
Configuration Description
The following screens will guide you in selecting a custom configuration for your method.
< Back
Next >
Cancel

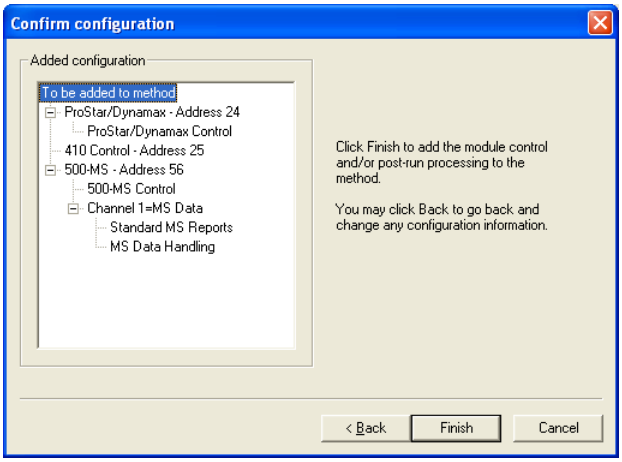
Select Instrument 1 to add automatically the hardware modules currently configured in System Control and click **Next**.



Select the channels for post-run processing.

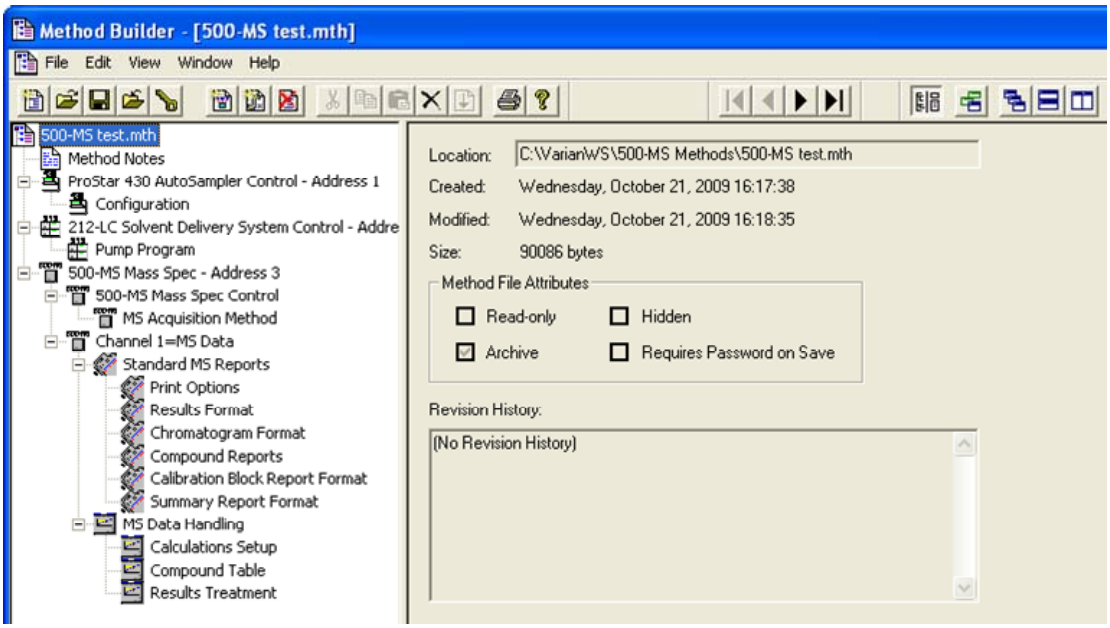


After you select the data channels and type(s) of post-run processing for each detector, the wizard creates a method. The method sections contain default values for all parameters, which you can edit. Data handling and reports are discussed in the MS Workstation Software Reference Manual.


Confirm configuration
Added configuration
To be added to method
ProStar/Dynamax - Address 24
ProStar/Dynamax Control
410 Control - Address 25
500-MS - Address 56
500-MS Control
Channel 1=MS Data
Standard MS Reports
MS Data Handling
Click Finish to add the module control and/or post-run processing to the method.
You may click Back to go back and change any configuration information.
< Back
Finish
Cancel

Method Builder Window

Overview



The Method is displayed in two panes. The Directory pane on the left is an outline of the Method. Click an item in the Directory to change the content of the Parameters pane on the right. Click and drag the splitter bar to resize the panes.

To hide the Directory, click **Show/Hide Method directory** on the Window toolbar.

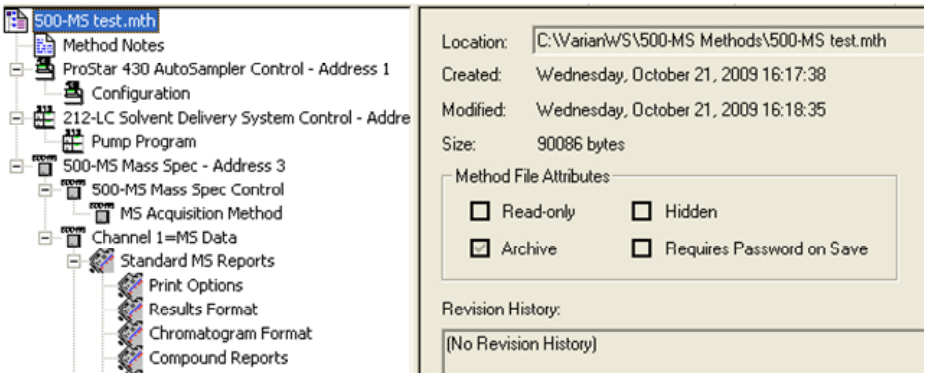
The menus at the top of the screen are: File, Edit, View, Window, and Help. Click each to see its contents. Below these menus, there are three toolbars: the Main toolbar opens, saves, and prints Methods; the Directory toolbar traverses the Directory outline; and the Window toolbar positions Method windows. Hold the mouse cursor over each button to see a Tooltip describing its function. A status bar is at the bottom of the Method Builder window. The toolbars and the status bar may be hidden or shown using the View menu.

Use the Method Builder to display and edit multiple Method files. If the Method Builder is already displaying one method, you can open another using the ***File… Open*** command. The Methods can be selected, cascaded, or tiled to compare the same sections in different Method files from the window menu on the window toolbar.

Method Parameters

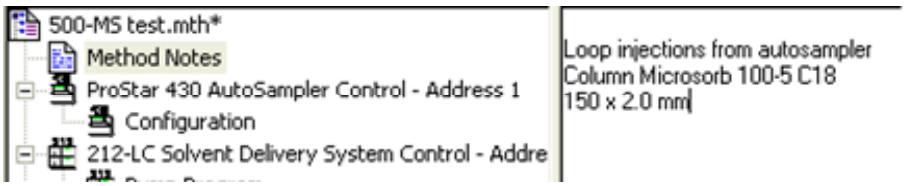
The Parameters pane displays the content of the method section selected in the Directory. Four types of windows can be displayed in the Parameters pane: method file information, method notes, hardware module descriptions, and editable parameters for the instrument modules, data handling, and reports.

File Information



Selecting the file name at the top of the Directory displays method related information, including name and directory path, creation and modification dates, file size, file attributes, and the revision history.

Method Notes



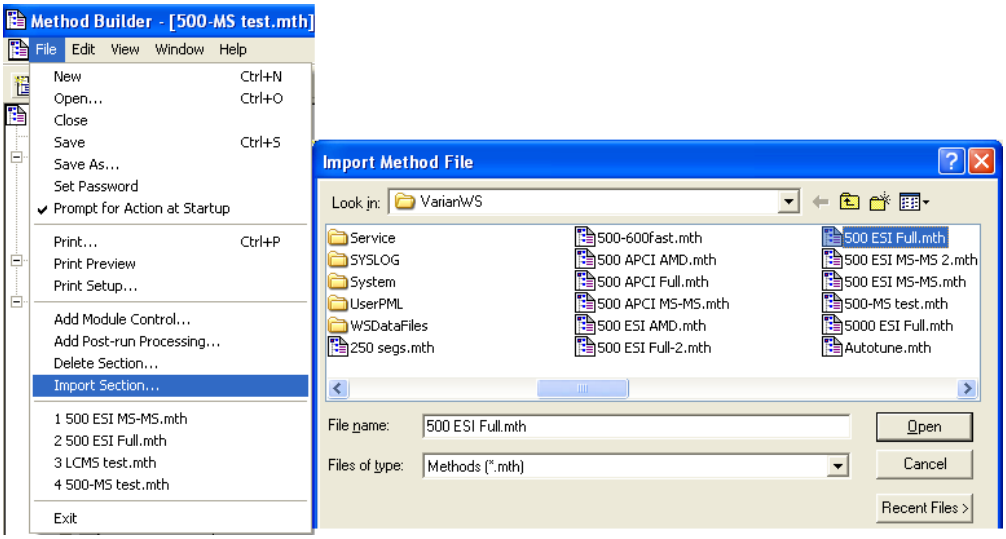
Selecting Method Notes displays a blank Parameters pane where you can enter notes about the method. You can enter information about the method, sample preparation, laboratory practices, and so on. It is possible to copy text from a Microsoft Word or Excel document and paste into the Method Notes; however, you cannot copy and paste graphics or icons this way. Method Notes are printed when the Method is printed.

Instrument Modules

Highlighting low-level outline branches for the 500-MS and the other LC hardware modules (those that cannot be expanded) displays Parameter panes with editable parameters for controlling the instrument, acquiring data, and post-run data processing and reporting.

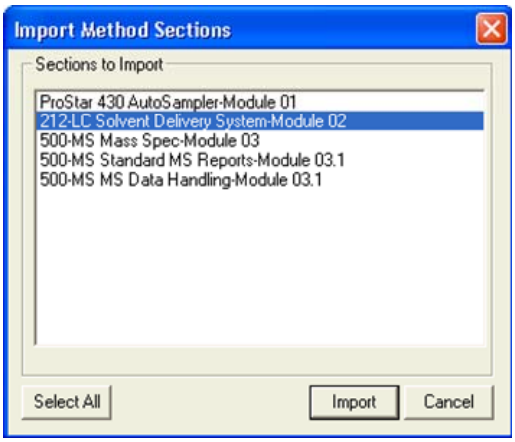
Importing Method Sections

You can copy sections from one Method to another. Open the target Method file. This is the Method to which you want to Import one or more sections. Use the **File>Import Section** command to open the Import Method File dialog.



Select the source Method from which you will import. Click the sections that you wish to import. If you click one section and then hold down the shift key while clicking on another section, all of the sections in between are selected. Hold the control key down while clicking on a section to add it to the selected files.

Clicking a highlighted section while holding down the control key removes that section from the list of selected files.

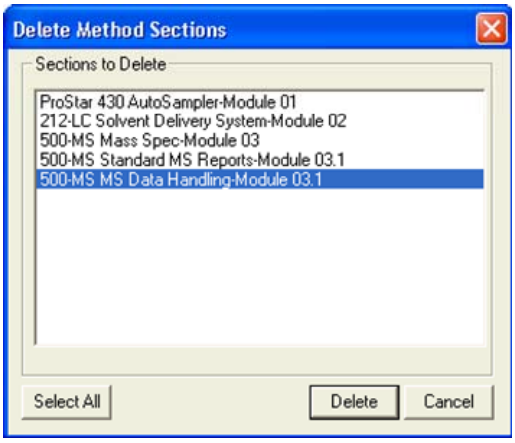


Click **Import** to import selected sections to the target Method. If the Method already has sections with the same module address and channel ID, you are prompted to assign a new module address and channel number to the imported section or overwrite the existing section in the current method.

Deleting Method Sections

Open the Method containing the sections you wish to remove. From the Method Builder Toolbar, click **Delete Section**. Or on the **File** menu, click **Delete Section**.

A dialog box displays the Method sections. Click the desired sections. Click one section and hold down the shift key while clicking another section to select a range. Hold down the control key while clicking a section to add that selection to the list. Click a selected section while holding down the control key to remove it.

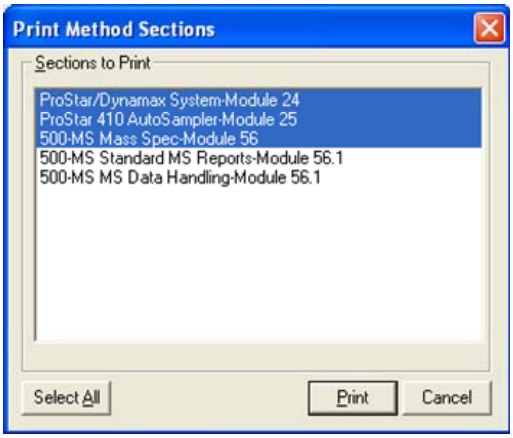


After highlighting the sections click delete to delete them. You are prompted for confirmation before each section is deleted.

Printing the Method

Click the **Print** icon on the Toolbar.

Select the desired Method sections, and click **Print**.



Password Protecting a Method

Click **Set Password** on the Method Builder Toolbar or use the command ***File… Set Password*** to open the Add Password dialog.

Enter the password and then re-enter it to confirm.

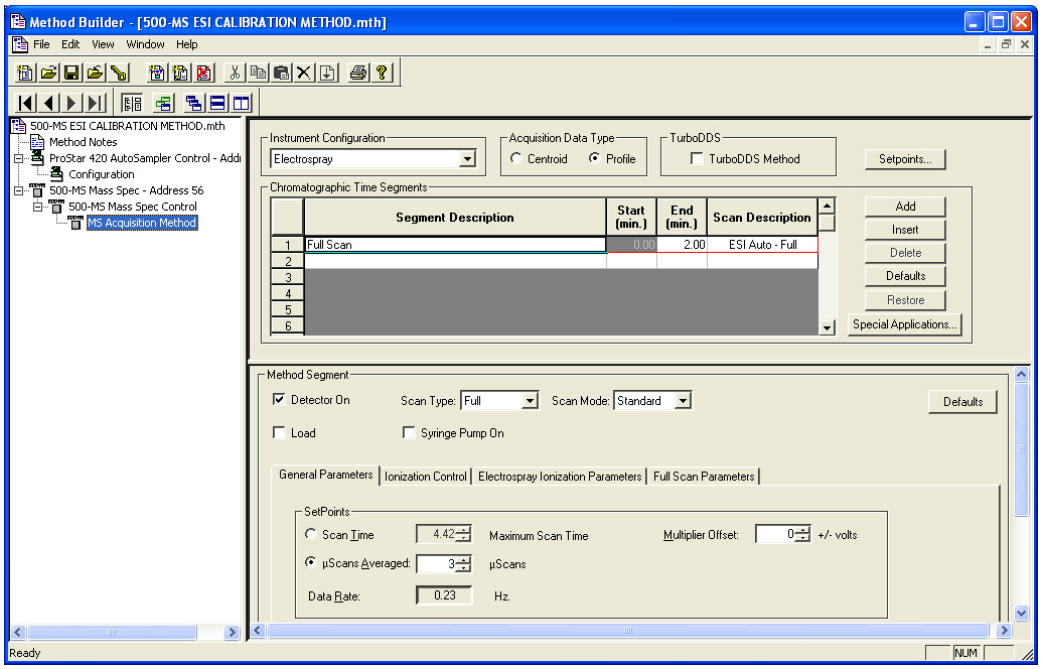
After a Method is password protected, it can be used in System Control for instrument control, data acquisition and data handling. It can be viewed in Method Builder as well.

However, the selected password must be entered to save Method changes. It is now “locked” and can be edited only when the password is known.

500-MS Method Section

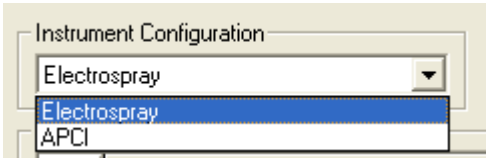
Setting Up the MS Method Editor Screen

In the Directory, point to **500-MS Mass Spec Control** and click **MS Acquisition Method**. The Parameters pane shows the MS acquisition method.



Setting the MS Instrument Configuration

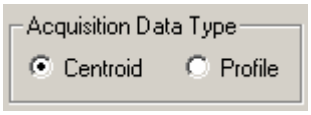
Most of the following configuration settings are for the entire method and are not time segment variables.



Configuration Menu

There are two hardware configurations: Electrospray (ESI) and Atmospheric Pressure Chemical Ionization (APCI). If you change the ionization configuration in a method, all the previous Time Segment information is replaced with default values.

Choosing the MS Acquisition Data Type



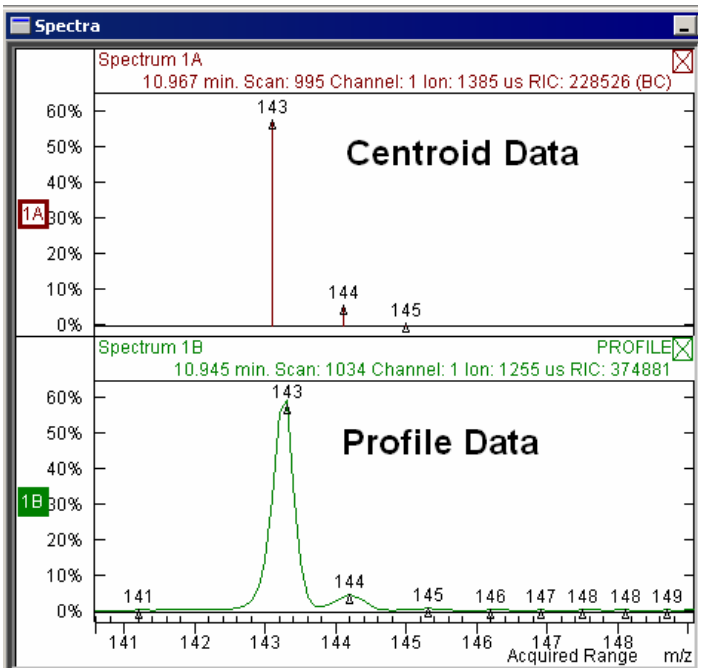
Either Profile or Centroid data may be acquired in a method.

Centroid Data

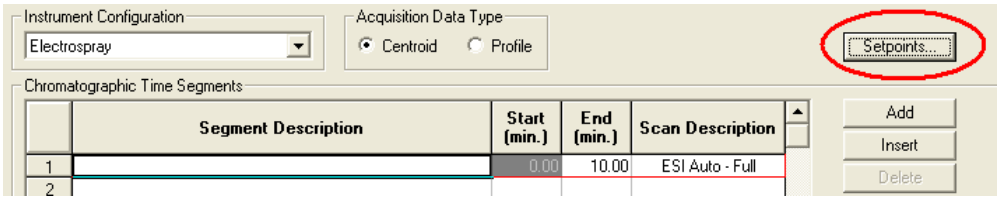
The standard data acquisition type is Centroid; in this approach, the analog signal from the detector is processed by the analog to digital converter. The centroid is the center of gravity of each mass peak that is reported to one decimal place, that is, m/z 231.2. Centroid data are required for library searches and quantitation.

Profile Data

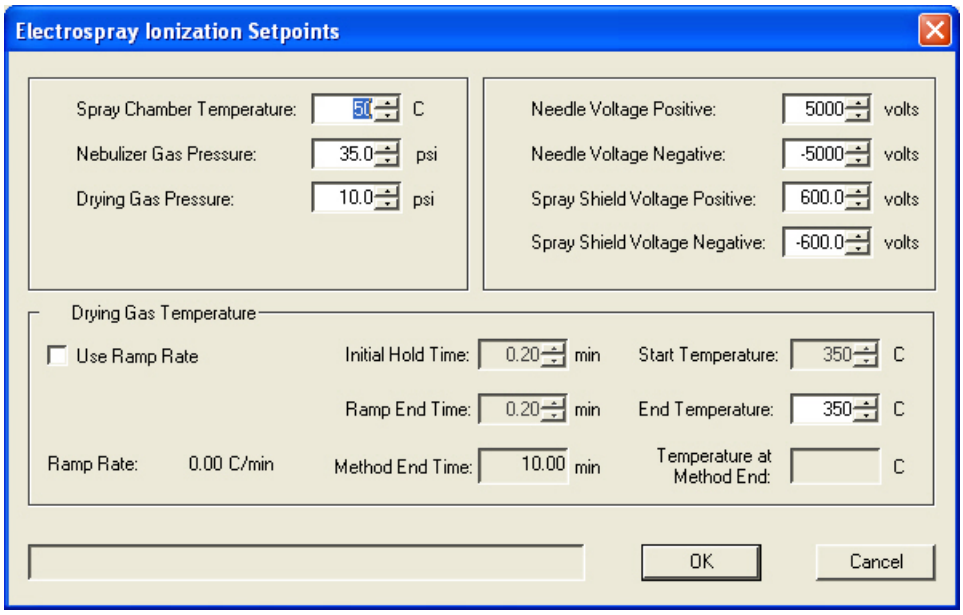
Profile data is raw data. The data files are much larger than centroid because ten data points are collected at each m/z. Profile data can be used to diagnose difficulties with mass resolution or mass assignment. Profile Data is used to determine molecular weight of voltage charged molecules. The following shows centroid data in the top and profile data in the bottom. Profile data can be quantitated.



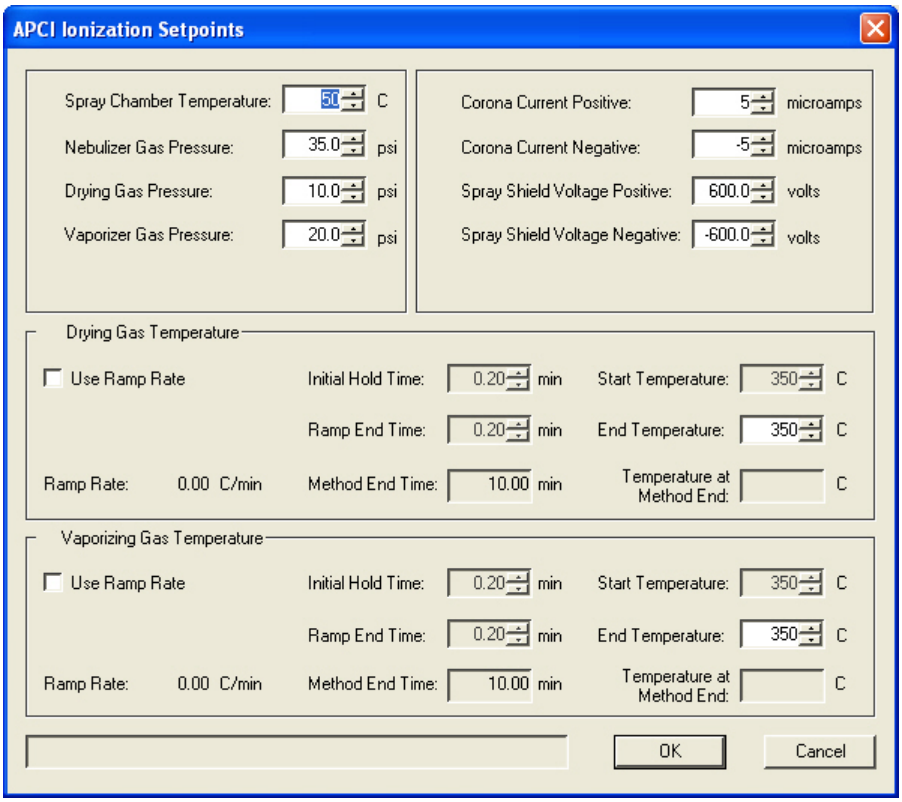
Setting Method Setpoints



NOTE: All the set point parameters can be changed per segment except for the temperature ramp rates for Drying Gas and Vaporizing Gas (APCI). They are adjusted in the Ionization Parameters tab.



NOTE: An explanation of the Drying Gas Temperature ramp rate follows the APCI Setpoints screen capture.


APCI Ionization Setpoints
Spray Chamber Temperature: 50 C
Nebulizer Gas Pressure: 35.0 psi
Drying Gas Pressure: 10.0 psi
Vaporizer Gas Pressure: 20.0 psi
Corona Current Positive: 5 microamps
Corona Current Negative: -5 microamps
Spray Shield Voltage Positive: 600.0 volts
Spray Shield Voltage Negative: -600.0 volts
Drying Gas Temperature
Use Ramp Rate
Initial Hold Time: 0.20 min
Start Temperature: 350 C
Ramp End Time: 0.20 min
End Temperature: 350 C
Ramp Rate: 0.00 C/min
Method End Time: 10.00 min
Temperature at Method End: C
Vaporizing Gas Temperature
Use Ramp Rate
Initial Hold Time: 0.20 min
Start Temperature: 350 C
Ramp End Time: 0.20 min
End Temperature: 350 C
Ramp Rate: 0.00 C/min
Method End Time: 10.00 min
Temperature at Method End: C
OK
Cancel

Drying Gas Temperature Ramp (ESI and APCI)

In gradient applications that start with very low aqueous mobile phase and end with very high aqueous phase, you can ramp the temperature of the drying gas.

Setting the drying gas temperature ramp:

1. Click **Use Ramp Rate** to enable it.
    a. Enter an **Initial Hold Time**, if needed.
    b. Enter the Start Temperature.
    c. Enter the Ramp End Time.
    d. Enter the End Temperature.

Vaporizing Gas Temperature Ramp (APCI only)

In gradient applications that start with very low aqueous mobile phase and end with very high aqueous phase, you can ramp the temperature of the vaporizing gas.

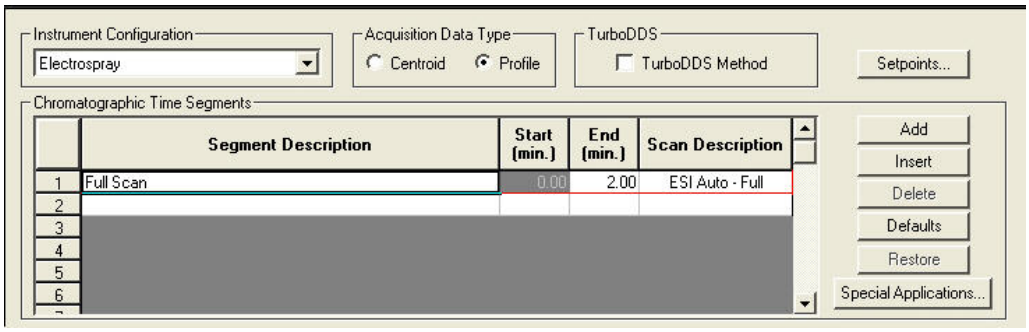
Setting the vaporizing gas temperature ramp:

1. Click **Use Ramp Rate** to enable it.
    a. Enter an **Initial Hold Time**, if needed.
    b. Enter the Start Temperature.
    c. Enter the Ramp End Time.
    d. Enter the End Temperature.

Editing Chromatographic Time Segments

NOTE: The following settings are not specific to the entire method. Each time segment can have individual settings.

You can program the mass spectrometer to acquire data differently by dividing the analysis into chromatographic time segments. The default method has a single time segment. Up to 250 time segments may be defined for a single analysis. The maximum run time is 650 minutes.



Click a row number to highlight it. Click **Add** to add a new segment with the same MS parameters of the last segment in the table. Click **Insert** to insert a new row before the currently selected row with the same parameter values. **Delete** deletes the currently selected row(s). **Default** inserts system default parameters to the current segment. **Restore** restores current segment parameters that were present when the method was first opened or last saved.

You can enter a user-selected segment description of up to 60 characters into the Segment Description field. Click the fields for the Start and End times and enter the desired values in minutes.

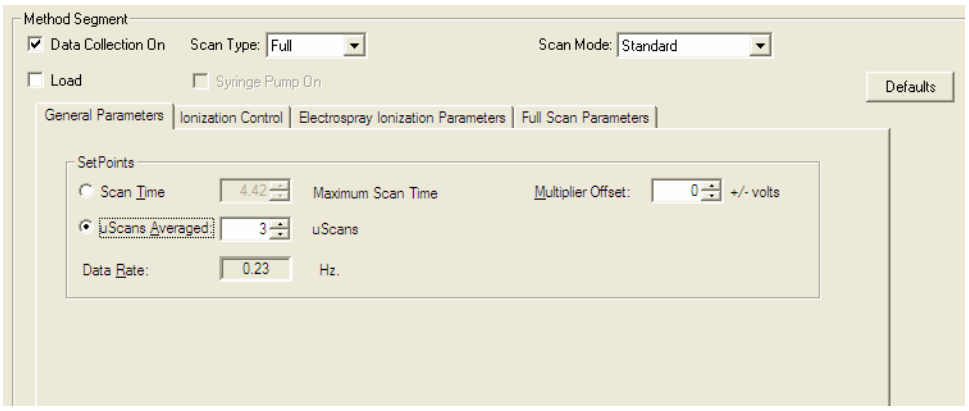
The Scan Description field is entered automatically based on the selection of Scan Type and Ionization mode in the Method Segment fields.

Chromatogram Time Gaps in Multi-Segment Methods

Depending upon the instrument configuration, you can acquire data using Scan Type ion preparation techniques. After multiple segments are acquired, MS Data Review displays each segment independently in the Plots View chromatogram display. There is no connection between the last point in one segment and the first point of the next segment. There is a small time gap between segments. This gap is less than one second.

Programming Individual Time Segments

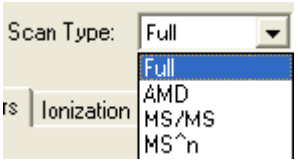
Selections across the top determine the available parameters.



Data Collection On

- If Detector On is checked, ions are detected for data acquisition.
- If Detector On is not checked, the multiplier is OFF during the segment and ions are not detected.

Scan Type



The scan type is either full-scan or a special ion preparation technique. The default is Full.

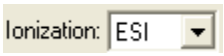
Full: Full-scan data for the selected mass range

AMD: Automated Method Development of MS/MS conditions. Up to ten voltages for collision-assisted dissociation of the MS/MS precursor ion can be tested on a single chromatographic peak using this feature. After the best dissociation condition has been identified it can be selected for MS/MS, MRM, or MS^n.

MS/MS: MS/MS, or tandem mass spectrometry, is the isolation of a precursor ion formed during ionization, dissociation of the precursor ion by applying energy, trapping and scanning the resulting product ions. Multiple Reaction Monitoring (MRM) can be performed for multiple precursor ions. This is useful when target analytes co-elute with deuterium-labeled internal standards of the same compound. It is also useful when target analytes elute too closely for different time segments to be constructed

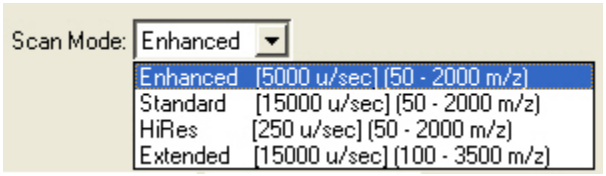
MS^n: Multistage MS/MS, a product ion from one tandem MS/MS experiment becomes the precursor ion for the next stage: MS^3, MS^4, up to MS^10.

Ionization



The available ionization mode depends on the attached device. ESI or APCI.

Scan Mode


Scan Mode: Enhanced
Enhanced [5000 u/sec] (50 - 2000 m/z)
Standard [15000 u/sec] (50 - 2000 m/z)
HiRes [250 u/sec] (50 - 2000 m/z)
Extended [15000 u/sec] (100 - 3500 m/z)

Enhanced: (Enhanced Resolution) A prescan in Automatic Gain Control mode is used to determine the optimum ionization time. Ions are scanned at 5000 µ/sec. Mass Range: 50-2000m/z. Scan a wide range and determine if an ion is doubly or singly charged.

Standard: The default. Default uses a prescan in Automatic Gain Control mode to determine the optimum ionization time. Ions are scanned at15000 µ/sec. Mass Range: 50-2000m/z.

NOTE: Use the Standard mode for most routine applications. The Standard mode has similar sensitivity to Enhanced mode and can provide more sample points across a chromatogram. Enhanced mode is best to use if you need maximal resolution (to observe multiply charged peaks for example).

NOTE: High Resolution and Extended Mass Range are available only with the TurboDDS™ option.

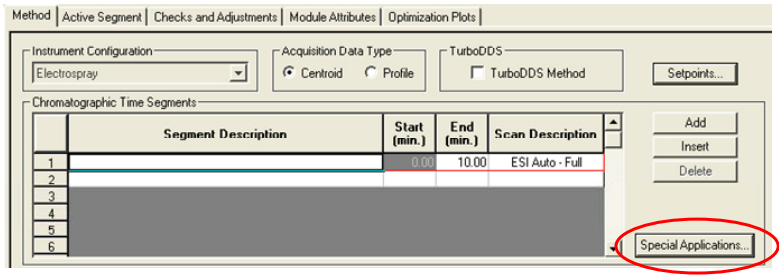
HiRes: (High Resolution) Scan a range of 10 m/z (from 50-2000 m/z) around the specified mass with a scan rate of 250 u/sec. The spectrum is useful for determining the charge state of a know ion by measuring the mass difference of the isotope peaks.

Isotope Mass Difference	Charge State
1.00	1
0.50	2
0.33	3
0.25	4

Extended: (Extended Mass Range) Scan from 100 to 3500 m/z at 1500u/sec. The mass resolution is lower than enhanced or standard.

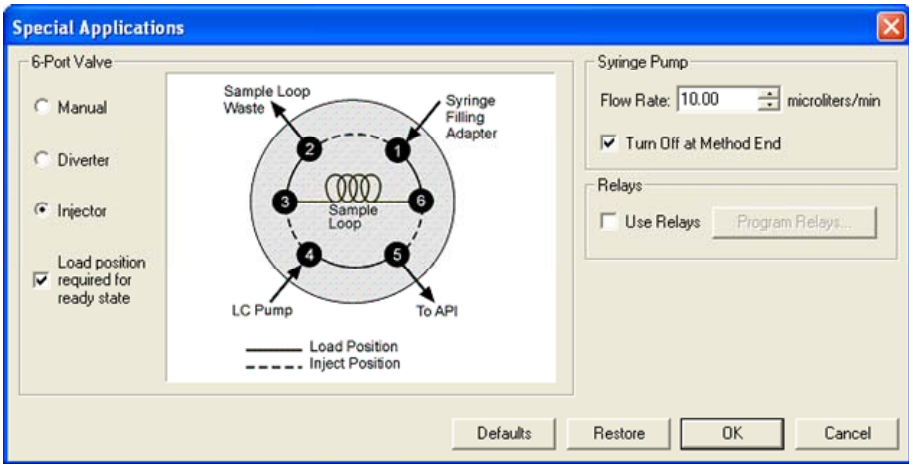
Special Applications

The **Special Applications** dialog box has controls for the 6-Port Valve, the Syringe Pump and Relays.

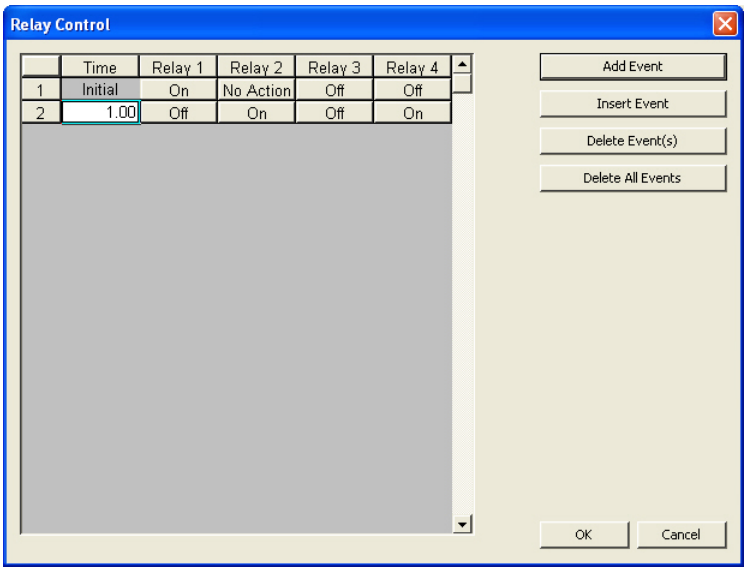


6-Port Valve: The selection affects the display in the Method Segment pane.

Syringe Pump: When the Syringe Pump is enabled in the Method Segment pane, enter the Flow Rate and if appropriate, click to turn the syringe pump off at the end of the run.



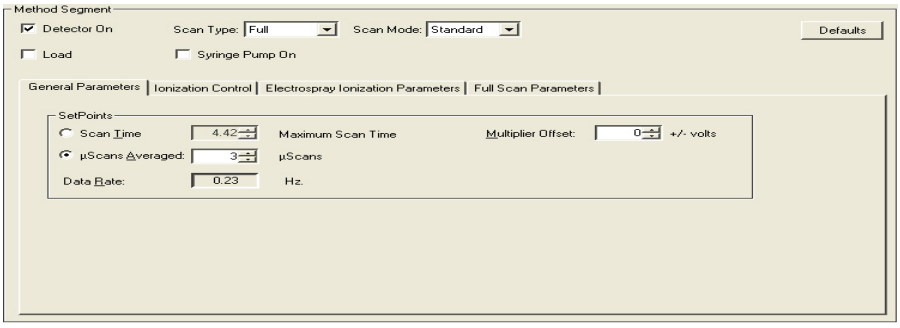
Relays: There are four TLL-compatible relays on the back of the 500-MS. They use a 9-pin “D” shell connector. Select **Relays** and click **Program Relays,** to open the **Relay Control** dialog. Control the relays using a time program.



ESI Parameters

This section details how to set parameters for an electrospray ionization method. In **System Control**, click **Active Segment**.

General Parameters



Scan Time, uScans Averaged, and Data Rate are linked. When you change the Scan Time, the Minimum number of Scans Averaged and the Data Rate are calculated for you. If you adjust the Minimum Number of Scans Averaged, the Scan Time and Data Rate are calculated for you.

The **Customize** button opens the **Count Threshold** Parameter.

There is a trade-off between how rapidly spectra are collected and the reproducibility of mass spectra or peak areas. The more microscans averaged, the more reproducible the mass spectrum. However, collecting more data points across a chromatographic peak improves peak area reproducibility for replicate injections.

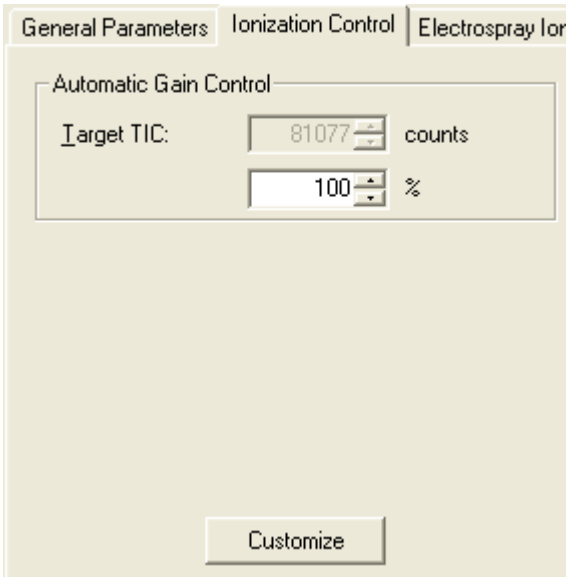
Averaging three scans provides good spectral quality and an acceptable data rate for most samples.

The Multiplier Offset adjusts the EM voltage as much as ±300V relative to the current multiplier setting in the Module Attributes tab dialog in Manual Control

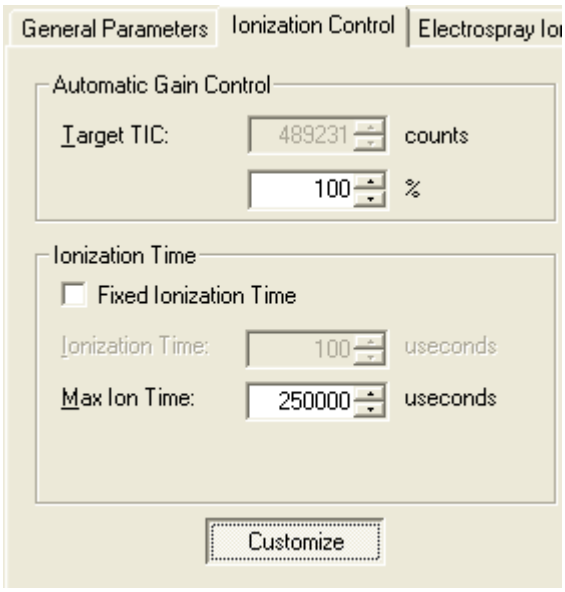
(this is usually the 10^5 gain value from Auto Tune). Sometimes better sensitivity is achieved, particularly in techniques such as MS/MS, when the multiplier voltage is increased. Note that this adjustment can be made on a segment-by-segment basis.

The Count Threshold is normally 1 count; a value of 2-3 counts reduces the number of low-level ions reported in the mass spectrum. This approach may improve library searches and reduce data file size at the cost of somewhat less detailed information in the mass spectra. The count threshold is shown only if the **Customize** button is selected.

Ionization Control



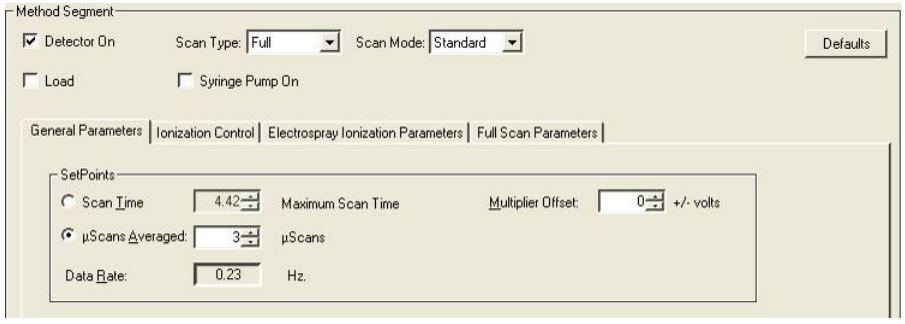
The Target Total Ion Current, or TIC, is adjusted in Ionization Control view. The Automatic Gain Control (AGC) algorithm uses the ion count from a prescan at fixed ion time, with the target value, to calculate the ion time necessary to fill the ion trap with the target number of ions. The objective is to fill the trap with an optimal number of ions during each analytical scan. In full scan mode, an optimal Target TIC is automatically calculated and adjusted depending upon the set scan range in the **Full Scan Parameters** tab (the calculated value for negative polarity is about 1/5 of positive polarity). In MS/MS, AMD and MS^n modes, the optimal Target TIC is fixed. This automatic setting can be overridden by entering the ***nnn*%** field. For example: To double the Target TIC use 200%; to halve the Target TIC use 50%). Do not set the Target TIC too high or spectral distortions due to space charge (loss of MS resolution and/or shift in mass assignments for strong chromatographic peaks) may result. Typically, the automatically calculated Target TIC at 100% gives the best results.



Use **Customize** to run fixed ion time experiments and/or to change the Maximum Ion Time for Automatic Gain Control. Fixed ion time experiments are generally diagnostic tests and are not used for qualitative or quantitative analysis.

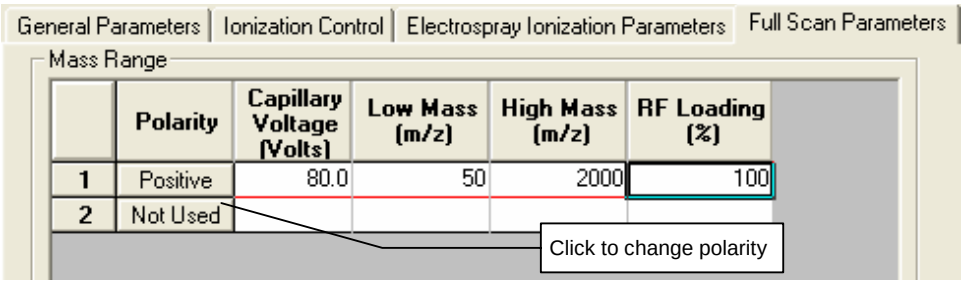
If the ion time in the baseline of the chromatogram is not reduced below the maximum ion time, the ion trap does not fill with enough ions to match the Target TIC. Changing the Maximum Ion Time can improve the sensitivity of techniques such as MS/MS. This parameter can be increased to 650,000 µsec, however, doing so reduces the data rate.

Electrospray Ionization Parameters



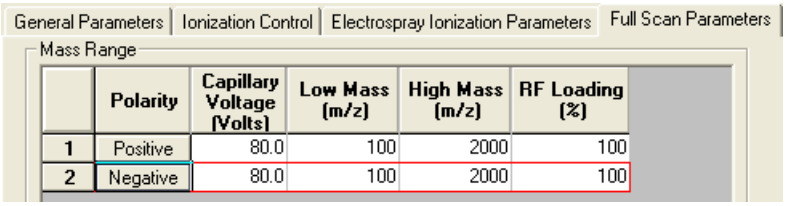
These default settings are suitable for many low flow rate applications. They can be changed for each segment.

Full Scan Parameters



Select the mass range for data acquisition for a segment. The default value for RF Loading% is 100. An optimum RF storage level is calculated automatically for the mass range that you select. However, you may customize the RF storage level by entering a percentage of this predetermined level. The polarity of the acquired ions can be selected here for each segment.

To do polarity switching for each segment, add a second scan channel to the segment by clicking **Not Used**. Enter the parameters for that scan and click **Not Used** until **Negative** is displayed.

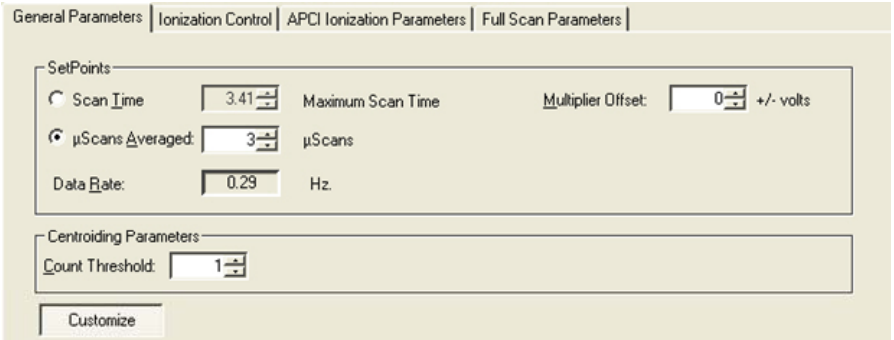


APCI Parameters

This section explains how to set parameters for an atmospheric pressure chemical ionization method.

General Parameters

Scan Time, uScans Averaged, and Data Rate are linked. When you change the Scan Time, the Minimum number of Scans Averaged and the Data Rate are calculated for you. If you adjust the Minimum Number of Scans Averaged, the Scan Time and Data Rate are calculated for you.



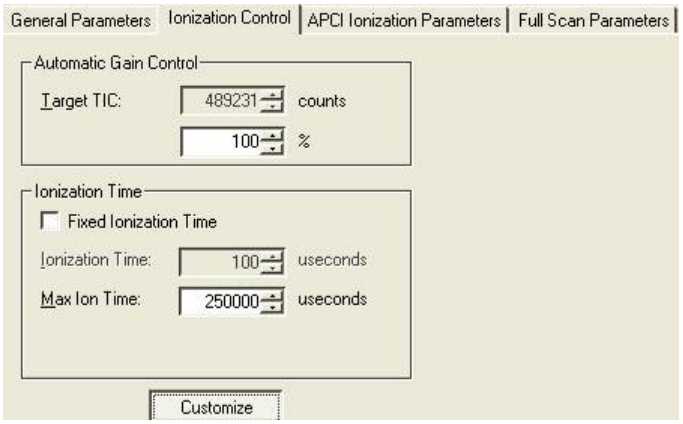
There is a trade-off between how rapidly spectra are collected and the reproducibility of mass spectra or peak areas. As more microscans are averaged, the relative intensities of ions in the mass spectrum become more reproducible. However, collecting more data points across a chromatographic peak improves peak area reproducibility for replicate injections.

Averaging three scans provides good spectral quality and an acceptable data rate.

The Multiplier Offset adjusts the EM voltage as much as ±300V relative to the current multiplier setting in the Module Attributes tab dialog in Manual Control (this is usually the 10^5 gain value from Auto Tune). Sometimes better sensitivity is achieved, particularly in techniques such as MS/MS, when the multiplier voltage is increased. Note that this adjustment can be different for each segment.

The Count Threshold is normally 1 count; a value of 2-3 counts reduces the number of low-level ions reported in the mass spectrum. This approach reduces data file size at the cost of less information. The count threshold is shown only if Customize is selected.

Ionization Control

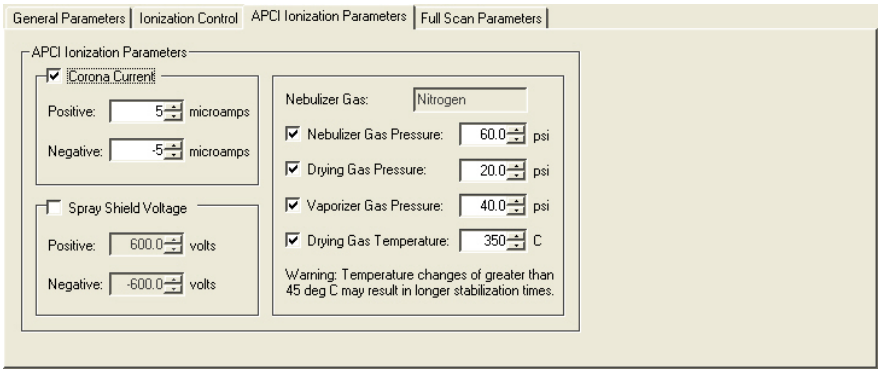


Set the Target Total Ion Current (TIC) or value in Ionization Control. The Automatic Gain Control (AGC) algorithm uses the ion count from a prescan at fixed ion time, along with this target value, to calculate an ion time necessary to fill the ion trap with the target number of ions during the analytical scan. The objective is to fill the trap with an optimal number of ions during each analytical scan. In full scan mode, an optimal Target TIC is automatically calculated and adjusted according to the set scan range in the Full Scan Parameters tab (the calculated value for negative polarity is about 1/5 of that for positive polarity). In MS/MS, AMD and MS^n modes, the optimal Target TIC is fixed. This automatic setting can be overridden by setting the nnn% field (for example, To double the Target TIC use 200%; to halve the Target TIC use 50%). The Target TIC should not be set too high or spectral distortions due to space charge (loss of MS resolution and/or shift in mass assignments for strong chromatographic peaks) may result. Typically, the automatically calculated Target TIC at 100% gives the best results.

Click Customize, to run fixed ion time experiments and/or change the Maximum Ion Time for Automatic Gain Control. Fixed ion time experiments are used as diagnostic tests and are not for qualitative or quantitative analysis.

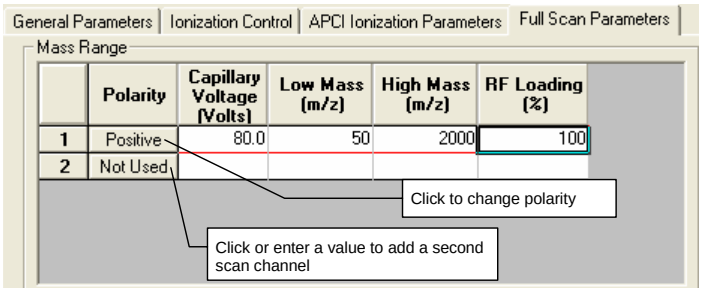
If the ion time in the baseline of the chromatogram is not below the maximum ion time, the ion trap does not fill with ions to the level designated by the Target TIC. Changing the Maximum Ion Time improves the sensitivity of techniques such as MS/MS. This parameter can be increased to 650,000 µsec, doing so reduces the data rate.

APCI Ionization Parameters


General Parameters
Ionization Control
APCI Ionization Parameters
Full Scan Parameters
APCI Ionization Parameters
Corona Current
Positive: 5 microamps
Negative: -5 microamps
Spray Shield Voltage
Positive: 600.0 volts
Negative: -600.0 volts
Nebulizer Gas: Nitrogen
Nebulizer Gas Pressure: 60.0 psi
Drying Gas Pressure: 20.0 psi
Vaporizer Gas Pressure: 40.0 psi
Drying Gas Temperature: 350 C
Warning: Temperature changes of greater than 45 deg C may result in longer stabilization times.

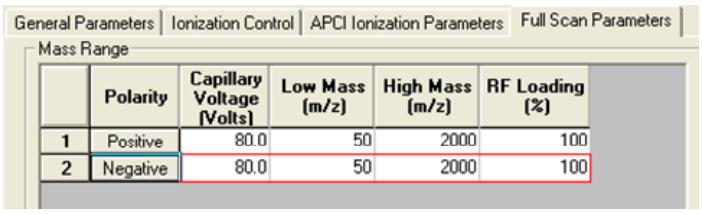
These settings are for the entire method and can be used for many applications. You can override and adjusted them for each segment.

Full Scan Parameters



Select the mass range for data acquisition for a segment. The default value for RF Loading% is 100. An optimum RF storage level is calculated automatically for the mass range that you select. However, you may customize the RF storage level by entering a percentage of this predetermined level. The polarity of the acquired ions can also be selected for each segment.

Polarity switching can be accomplished by adding a second scan channel to the segment by clicking **Not Used**.



TurboDDS™ Data Acquisition

Overview

NOTE: TurboDDS and High Resolution are only available if you have the TurboDDS chip installed.

Use Turbo Data Dependent Scanning (TurboDDS) to do automated MS^n scans on peaks as they elute from the column. You do not need to know the retention times or m/z values of the ions before making the injection. In a typical TurboDDS run, a survey scan is done followed by a high resolution scan and then multiple MS^n sequences are done. Please review the material in the preceding sections if you are not familiar with auto tuning, creating, and using methods.

Set parameters in the method to determine the number of levels of MS scans to do and the number of ions to be scanned. The survey scan, which is a full scan, is the first scan. Set threshold parameters and include/exclude rules to determine which ions from the survey scan will be precursor ions for the subsequent MS scan levels. Up to 5 levels of scans can be done. High resolution scans can be done on the ions that meet the triggering requirements.

Enter the parameters using the TurboDDS Parameters tab and the TurboDDS Trigger tab in the Method Builder

- TurboDDS parameters determine the number of ions to select and the number of MS scan levels done and the thresholds for each scan level.
- TurboDDS Trigger parameters determine
- How close the m/z values of ions can be before they are considered the same ion (isotopic peaks)
- How many times an ion is analyzed before going to the next most intense ion in the survey scan
- How specific ions in the survey scan are selected for subsequent MS scan levels.

Acquire a TurboDDS data file and open it in MS Data Review. MS Data Review has tools that simplify examining spectra. The Software Reference Manual has information about reviewing TurboDDS data.

TurboDDS Hints

Focus the survey scan in the mass range of interest. More scans are acquired across the chromatographic peak when the mass range is smaller.

Optimize the number of micro-scans.

- Using fewer micro-scans with narrow peaks allows more data points to be acquired across the peak.
- Using more micro-scans with wide peaks produces spectra with a higher S/N.

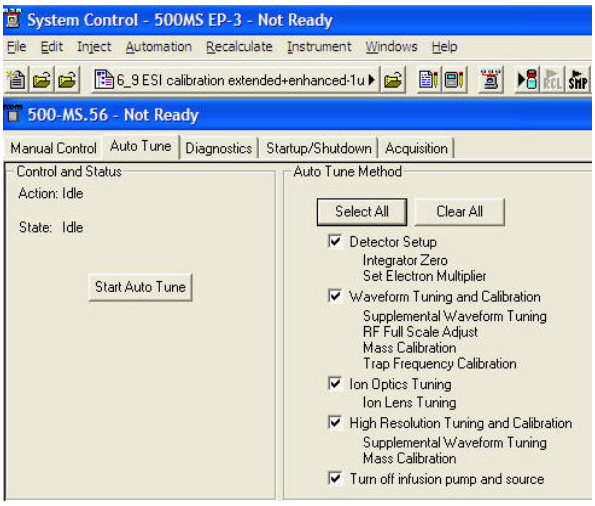
Use dynamic exclusion to ignore ions after they have been analyzed for several scans. MSn scans will be done on the ions of lesser intensity. If an ion may also be present later in the chromatogram, use dynamic exclusion to restore it after the set time. Occasionally, ions of similar heights may not be dynamically excluded as expected. Dynamic exclusion specifies that an ion will be excluded after a certain number of consecutive scans. If the most intense ion varies from scan to scan, the dynamic exclusion list may not be updated. If an ion is on the exclude list, it will always be excluded from TurboDDS. The exclude list takes precedence over the include list.

Auto Tune for TurboDDS™

Use the TurboDDS Tuning solution, part number 393211901.

Auto Tune for TurboDDS is similar to Auto Tune for non-TurboDDS applications. The Auto Tune Method section has

- Parameters in common with non-TurboDDS: Detector Setup, Waveform Tuning and Calibration, Ion Optics Tuning, Turn off infusion pump and source.
- Parameters unique to TurboDDS: High Resolution Tuning and Calibration



Method Builder for TurboDDS™

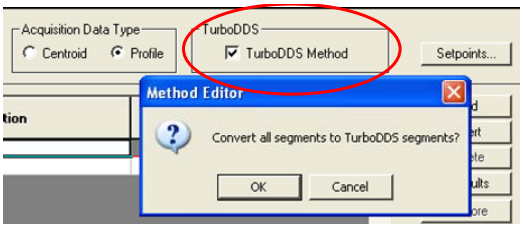
Enabling TurboDDS

In the **Method Builder** window, click **TurboDDS Method** to enable it.

The **Method Editor** dialog box appears. Click **OK** and all segments are converted to TurboDDS.

TurboDDS data can be acquired in either Centroid or Profile mode.

- Use High resolution data in profile mode to determine the charge state.
- Use centroid data to export protein data to external databases.



- Use multiple TurboDDS segments to change Trigger or TurboDDS parameters during the acquisition.
- Use either **Standard** or **Enhanced** Scan Mode.

TurboDDS Parameters Tab

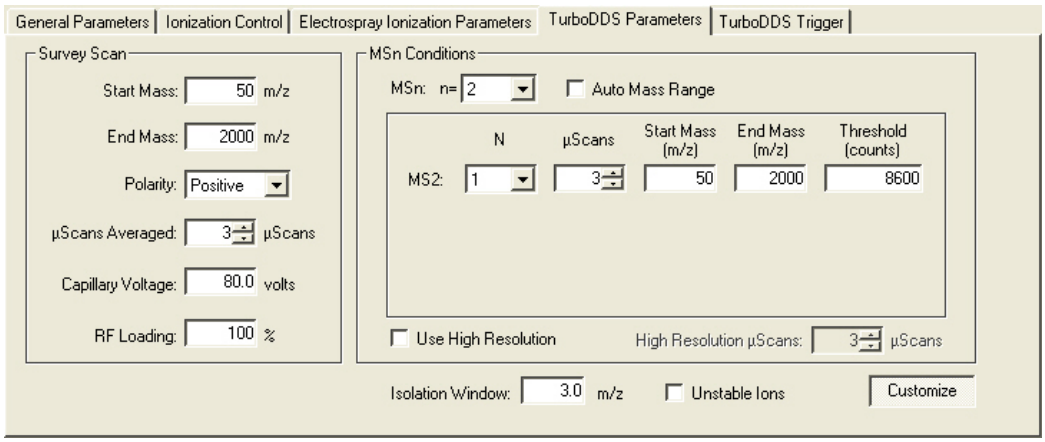
Enter parameters for the Survey Scan and MSn Conditions.

Survey Scan Parameters

- **Start and End Mass:** Range 50-2000 m/z
- **Polarity:** Select either Positive or Negative for all scans.
- **uScans Averaged:** Select from 1 to 9. Average more to generate spectra with better quality, but fewer spectra are produced. Fast chromatographic peaks may not be well resolved.
- **Capillary Voltage:** Enter from 0 to 300V. The sign is set to match the polarity selected.
- **RF Loading:** Enter from 0 to 300%. Sets the trapping conditions as the ions enter the trap.
- For full scan applications, enter 100% to let the software set the optimum value.
- For target analysis, optimize the RF loading % for each compound to increase sensitivity.

MSn Condition Parameters

- **n:** Select from 2 to 5 to determine the number of MS level scans. If 3 is selected, after the survey scan is done, MS2 is done on ions that meet the trigger criteria. MS3 is done on product ions of the MS2 scans that meet the trigger criteria.
- **Auto Mass Range:** When enabled, the mass range used to detect product ions is determined automatically.
    - **Singly charged ions:** Start m/z is determined by the RF storage level and the End m/z is the precursor ion m/z plus 20.
    - **Doubly charged ions:** Start m/z is determined by the RF storage level and the End m/z is twice the precursor ion m/z plus 20.
- **uScans:** Select from 1 to 9 to determine the number of scans that are averaged for that MS level.
- **N:** Number of ions from the previous MS scan level that will be scanned for the current MS scan level. For example, if N is 3 on the MS2 line then 3 ions from the survey scan are used to generate MS2 spectra.
- **Start and End Mass:** If Auto Mass Range is not enabled, enter the start and end m/z value. (see Auto Mass Range). The product ions of these TurboDDS scans must be in specified scan range to be observed.
- **Threshold:** Ions that exceed the threshold number of counts and meet the trigger criteria are scanned at the next MS level.
- **Use High Resolution:** When enabled, a high resolution scan is done for each ion from the Survey Scan that meets the criteria. In the profile mode, you can determine up to 4 positive charge states by measuring the m/z difference between isotopic peaks.
- **High Resolution uScans:** Select from 1 to 9**.** Enter the number of uscans used to average each High Resolution analysis.
- **Customize:** Click Customize and two options appear.
- **Unstable ions:** Select to decrease the possibility of accidentally dissociating less stable precursor ions in the isolation step.
- **Isolation Window:** Select from 1 to the maximum window. The maximum window size is a function of the precursor mass and ranges from 3 to 14 m/z. The wider the isolation window, more ions remain in the ion trap after the isolation step and the intensity for less stable precursor ions improves.



TurboDDS Triggers Tab

Enter parameters for Trigger and Include/Exclude Conditions.

Trigger conditions

- **Threshold:** The value selected on the MS2 line in the TurboDDS Parameters tab is copied to this section. If you change the value on one tab, the other tab updates to that value automatically. Ions that have an intensity that is greater than the Threshold will initiate additional scans. After the most intense ion is analyzed, additional ions up to the N specified are scanned.
- **Minimum Intensity Ratio:** Select from 1 to 100%. This is an additional parameter for setting the threshold when N is greater than 1. Use it to trigger on ions that are more intense than the threshold and the specified percentage of the most intense peak in the survey scan. Increasing this value helps to remove noise spikes, if they appear to be selected, for data dependent actions, especially near the apex of a chromatographic peak.
- **Isotopic Equivalence:** Select from 1 to 10 m/z. Select a value to prevent more than one peak in an isotopic cluster from triggering TurboDDS scanning. This is helpful when isotope peaks of one ion are more intense that the base peak of another.

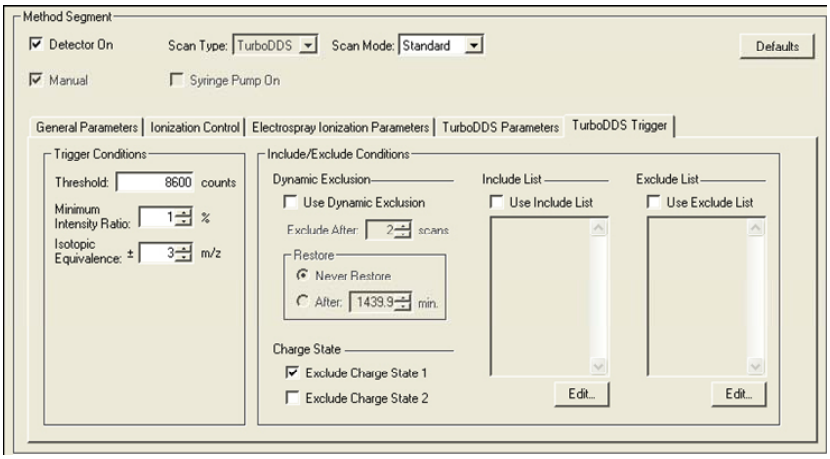
Include/Exclude Conditions

Use this section to determine which ions are subjected to data dependent scanning. The sections are Charge State, Dynamic Exclusion, Include List, and Exclude List.

- **Charge State:** The options are
- **Exclude Charge State 1:** Ions with a charge state of 1 are excluded. This is useful for applications such as peptide digests.
- **Exclude Charge State 2:** Ions with a charge state of 2 are excluded. This is useful for applications such as drug metabolites.
- **Dynamic Exclusion:** After an ion has been analyzed, you can eliminate it from further data dependent actions either for a set time

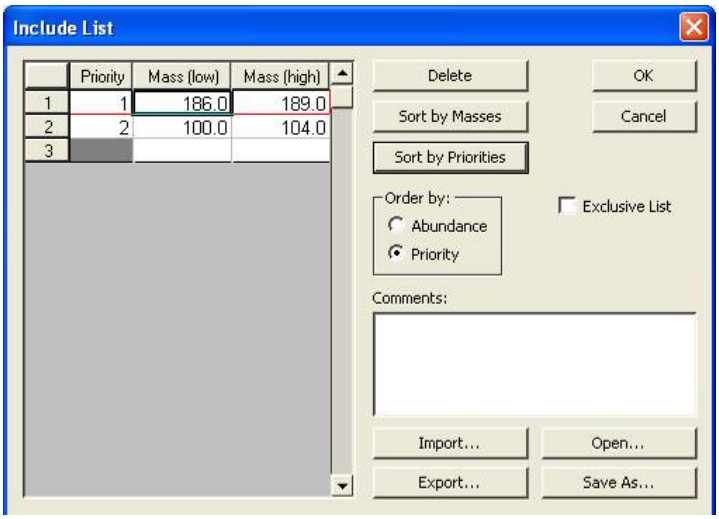
or for the rest of the run. Excluding these ions allows for the analysis of less intense ions.

- **Use Dynamic Exclusion Check Box:** Select to apply.
- **Exclude After n scans:** Select from 1 to 9 scans. After an ion is observed for the specified number of consecutive scans, it is added to the dynamic exclusion list. No further High Resolution or MSn experiments are done on it.
- **Restore:** The options are
  - **Never Restore:** Keep the ions on the dynamic exclusion list for the rest of the run.
  - **After n min.:** Remove the ions from the dynamic exclusion list after the specified time. This is useful for excluding ions from a chromatographic peak and searching for them in other peaks.



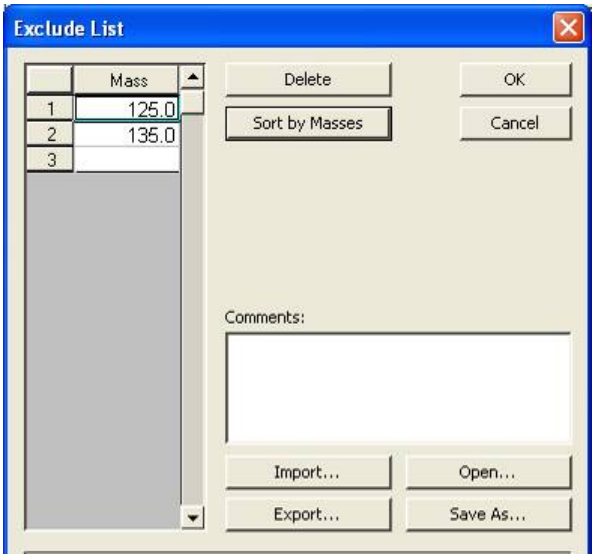
- **Include List:** You can use the Include List as an Exclusive List or a Non-exclusive List. Click Edit to open Include List. Activate the list by clicking the checkbox.
- **Exclusive List:** The listed mass ranges that exceed the threshold are the only ones searched. This ensures that the target ions, even if they have low abundance, are selected. Ions not on the list are displayed only on the survey scan, regardless of how intense they may be. Select ions in order of abundance or priority.
- **Non-exclusive List:** The software searches for the listed mass ranges that exceed the threshold. If no listed ions are found, other ions in the survey scan are selected. This ensures that in addition to possible low abundant target ions other ions from the survey scan are analyzed if the listed ions are absent. Select ions in order of abundance or priority.
- When Exclusive List is not enabled, the listed ions are searched for first and then ions that are not on the list are searched according to their intensities.
- **Low Mass, High Mass:** Select from 50.0 to 2000.0 m/z**.** Enter the mass range to search. The Low Mass must be lower than or equal to the high mass and overlaps are not permitted among listed ranges.

- **Priority:** As you enter the mass ranges, you can assign a priority to that range. When Order by Priority is selected, the mass ranges are searched according to the listed priority.
- **Order by:** Specifies the order the ions from the include list are used when the method is run. The options are:
  - **Abundance:** The most intense ion in the survey scan that meets the trigger criteria is analyzed first, followed by the next most intense ion, until N ions are analyzed.
  - **Priority:** The ions are searched for according the Priority assigned in the List, regardless of intensity. Only those ions that meet the trigger criteria are analyzed.
- Use the following commands with the Include List.
  - **Delete:** Click the row to be deleted, and then click **Delete**.
  - **Sort by Masses:**
    - Click to sort the mass ranges in order of increasing mass.
    - Click again to sort the mass ranges in order of decreasing mass.
  - **Sort by Priorities:**
    - Click to sort the mass ranges in order of increasing priority.
    - Click again to sort the mass ranges in order of decreasing priority.
  - **Comments:** Enter comments that are displayed each time you use the list.
  - **Import/Export:** Use other programs, such as a spreadsheet or a text editor to store or edit your lists. The file name extension is .txt. Only the information on the spreadsheet (mass range and priorities) is saved.
  - **Open/Save As:** Save Include lists or open them. The files name extension is .inc and all other information is included, such as the Comments, “Order by” selection, and if the list is exclusive.



- **Exclude List:** Create a list of ions that are not searched. Eliminate known background or contamination ions. Click Edit to open Exclude List. Activate the list by clicking the checkbox.
- Use the following commands with the Exclude List.
- **Delete:** Select the row to be deleted, and then click **Delete**.
- **Sort by Masses:**
  - Click to sort the mass ranges in order of increasing mass range.
  - Click again to sort the mass ranges in order of decreasing mass range.

  Note: Because these ions are always excluded, the sort order is not important. When the Exclude List is not used, the sort order is from low to high m/z.
- **Comments:** Enter comments that are displayed each time you use the list.
- **Import/Export:** Use other programs, such as a spreadsheet or a text editor to store or edit your lists. The file name extension is .txt. Only the masses are saved.
- **Open/Save As:** Save Include lists or open them. The files name extension is .inc. The **Comments**, and **Order by** entries are included.



Using APCI

Tuning

Tune and mass calibrate the 500-MS mass spectrometer using the Electrospray Ionization (ESI) chamber with the 500-MS Tune Solution (part number 0393210201). Use the ESI tune file for acquiring APCI data. For specifics regarding tuning, refer to the Tuning section of the 500-MS manual.

Optimization

The detector response varies for each type of analyte and depends upon the mobile phase composition and flow rate. Usually analytes are introduced by flow injection or infusion. Use the syringe pump at less than 100 µL/min. Adjust nitrogen flows and temperatures while performing flow injection or infusion of the analyte to achieve maximum signal to noise. At low flow rates, there can be arcing to the shield.

The most significant parameter is the vaporizer temperature. Its optimum value is a function of the sample (experimentally determined), the mobile phase composition (more water requires a higher temperature, while a higher percentage of organic solvent requires a lower temperature), and the flow rate (a higher flow rate requires a higher temperature). If the temperature is too low, droplets are observed at the outlet of the vaporizer tube. If the temperature is too high, the sample could thermally degrade.

If the gas flows is too low, the baseline may be unstable. If the gas flows is too high the size of the peaks may be reduced.

The vaporizer can be moved back in the spray chamber to lessen the likelihood of fouling the vacuum capillary when the matrix contains a large amount of nonvolatile material (but at some expense in signal intensity). To adjust the position of the vaporizer relative to the capillary entrance, loosen the four mounting screws on top of the spray chamber and slide the vaporizer assembly forward or backward. A numbered scale on the right side of the vaporizer assembly helps to reset the position. Be sure to tighten all four screws snugly when you are finished to avoid heat damage to the vaporizer. It must be firmly attached to the spray chamber for proper heat sinking. The following lists typical APCI operating parameters.

APCI Typical Operating Parameters

The APCI Source may become hot during operation. Take appropriate precautions.

Drying Gas: 20 psi at 150 °C

Nebulizing Gas: (60 psi): Nitrogen in positive mode, Air in negative mode (automatically switched)

Auxiliary Gas: 40 psi: as hot as possible without decomposing sample (maximum temperature setting is 550 °C)

Corona Current: 5-10 µA

Housing Temperature: 65 °C

Comparison of Parameters for ESI and APCI

PARAMETER	ESI	APCI
Drying Gas	20 psi @ 350 °C	20 psi @ 150 °C
Nebulizing Gas	60 psi	60 psi
Auxiliary Gas	N/A	40 psi @ 550 °C
Inner Needle	Adjustable	Fixed
Needle Assembly Up/Down	Adjustable	Fixed
Needle Assembly Translation	Adjustable	Adjustable
Needle Voltage/Current	5000V	5 µA
Optimum Liquid Flow Rate	200-400 µL/min	0.5-1 mL/min
Housing Temperature	40 °C	65 °C

MS/MS - Tandem Mass Spectrometry

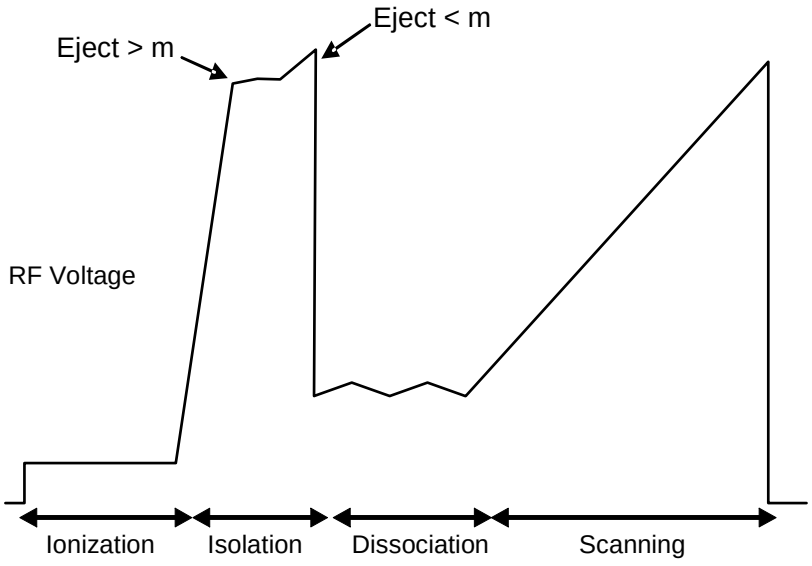
Overview

MS/MS is the isolation of a precursor ion formed in the API interface, dissociation of the precursor ion by energetic collisions with helium atoms, and trapping and scanning of the resulting product ions.

The ion trap MS/MS process is:

- Filling the ion trap with the selected precursor ion;
- Forming the product ion spectrum to obtain qualitative structural information to obtain qualitative structure information; and
- Increasing the signal-to-noise ratio by eliminating interfering matrix ions in the product ion spectrum through isolation and dissociation - the only ions in the product ion mass spectrum originate from ions that fall into the isolation window.

The MS/MS Scan Function



MS/MS Scan Function (Analytical scan only)

Product ion MS/MS is a four-step process.

1. The sample is ionized and the ion trap fills with the optimal number of ions. This is similar to full scan.
2. The selected precursor ion is isolated from all the unwanted ions.
3. The range of motion of the selected ions is increased, which results in energetic collisions. The selected ions fragment.
4. The fragment ions are ejected from the trap and scanned.

For MS^n, isolation and dissociation steps are carried out serially and the resulting ions are scanned to create the spectrum. While each step reduces the overall signal, the signal from unwanted ions decreases faster than the signal from the target ion.

Ion Formation and Matrix Ion Ejection

Ionization of the sample and any co-eluting matrix occurs in the API interface. A notched waveform may be applied to the end caps of the ion trap to eject resonantly ions above and below the specified precursor ion mass. This may or may not improve the performance of the instrument, depending on the sample composition. The waveforms are most effective when there is a large amount of matrix interference in the low mass range. Removal of most of the space charge from the trap improves mass resolution in subsequent MS/MS steps. The ions remaining in the trap include the optimum number of precursor ions, and ions that are in the coarse isolation window.

Precursor Ion Isolation

The precursor ions are then isolated from remaining matrix ions in a two-step process.

In the first step, a broadband waveform is applied to the trap to eject simultaneously all masses above the precursor ion mass. Isolation occurs at elevated RF levels where mass resolution is optimal. Thus, it is possible to achieve unit mass isolation over the entire mass range of the ion trap. This may be useful in isolating a single mass in an isotopic cluster, or in separating the precursor ion from interfering matrix ions.

In the second step, the ions with masses below the precursor ion mass are ejected by ramping the RF amplitude while applying the supplemental waveforms to the end cap electrodes. All ions having masses up to, and including, the mass just below the precursor ion mass are resonantly ejected. The supplemental waveforms are then turned off.

Product Ion Formation

Precursor ions are transformed into product ions by collision-induced dissociation (CID). The precursor ions constantly collide with helium gas in the ion trap. Normally these collisions involve relatively small energies, but if the translational energy of the precursor ion is increased, the collisions may convert the translational kinetic energy into internal vibrational energy.

If the precursor ion acquires enough vibrational energy, one or more chemical bonds in the ion may be broken, and in the case of singly charged ions, forming ions of lower m/z than the original precursor ion. If the precursor ion is multiply

charged, then the product ions may be observed throughout the mass range. The ion distribution that results from the CID process depends on the characteristics of the precursor ion and the amount of energy that has been converted into internal vibrational energy.

The amount of energy converted to internal energy in the precursor ion depends on the number of collisions (excitation time), the relative energy of the collisions (CID excitation amplitude), and the rate at which internal energy is removed by collision deactivation (related to the excitation method). Collision-induced dissociation in the ion trap is always in competition with ion ejection. If the CID excitation amplitude selected is too large, the precursor ion is ejected to the trap electrodes before it can collide with background helium atoms. If the CID excitation amplitude selected is too small, the energy of the precursor ion will not exceed the internal energy threshold required to break the chemical bonds and form product ions. This is because energy is constantly being removed by low energy collision deactivation. Therefore, CID excitation amplitude and excitation time are used to optimize the CID process in conjunction with an appropriate choice of excitation method. When a precursor ion is selected in the CID method editor, default times and amplitudes are automatically filled in. The exact optimum for a specific compound can be found by using the optimization plots page.

The translational kinetic energy of the precursor ion can be increased using one of two methods. Each method requires application of a waveform to the ion trap. The waveform amplitude is the CID excitation amplitude, and the length of time the waveform is applied is the excitation time.

The first method involves *non-resonant excitation*. In this approach, a low frequency supplemental dipole field is applied to the end caps of the trap, resulting in an instantaneous change in the potential energy of the ion in the trapping field. (The dipole field is an electric field oriented along the axis of the trap.) The restoring force of the trapping field converts the newly increased potential energy of the ion into increased translational kinetic energy. A portion of this kinetic energy is then converted into internal vibrational energy upon subsequent collisions. This process is repeated during each oscillation cycle of the low frequency dipole field.

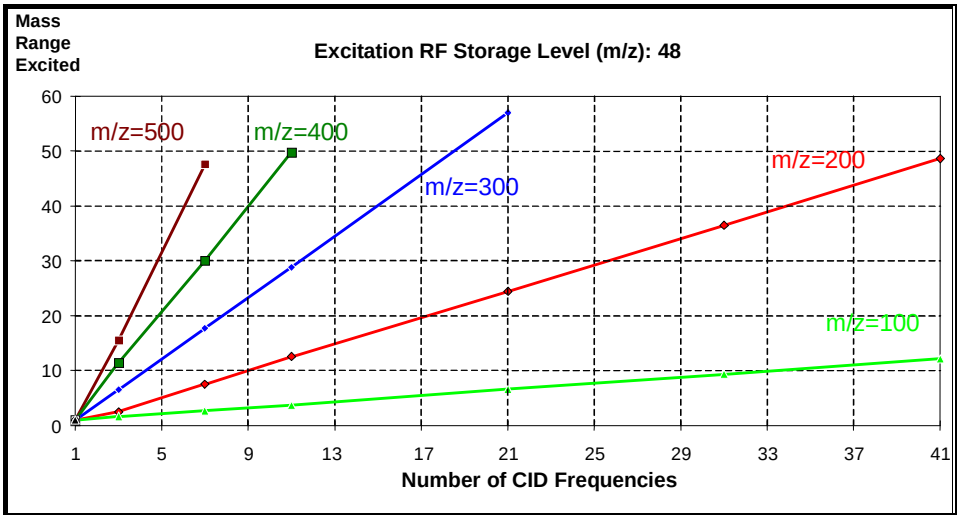
An advantage of using non-resonant excitation is that it is not critical to match the applied dipole frequency to the ion. Consequently, electronic drift, space charge effects, and sample concentration do not affect the method. This results in reproducible product ion spectra. This method is often useful with precursor ions that fragment by the breakage of a single weak chemical bond to form highly stable ions containing functional groups that do not undergo significant rearrangements. A disadvantage of the method is that it is not selective with respect to excitation of ions in the trap. Therefore, the method cannot be selectively tuned to excite only ions having a particular m/z. This method is less useful with precursor ions in which multiple chemical bonds are broken and with precursor ions that undergo complex rearrangements following collision-induced dissociation.

The second method for increasing the precursor ion kinetic energy involves *resonant excitation*. This method requires application of a high frequency supplemental dipole field to the end caps of the ion trap. The frequency must match the oscillation frequency of the trapped ion. The resonant frequency of the trapped ion depends on ion mass, space charge within the ion trap, RF trapping field amplitude, and other instrumental factors. Thus, it is difficult to calculate precisely its value. The amplitude of the RF trapping field is therefore modulated over a fixed range. Because the resonant frequency of the trapped ion depends

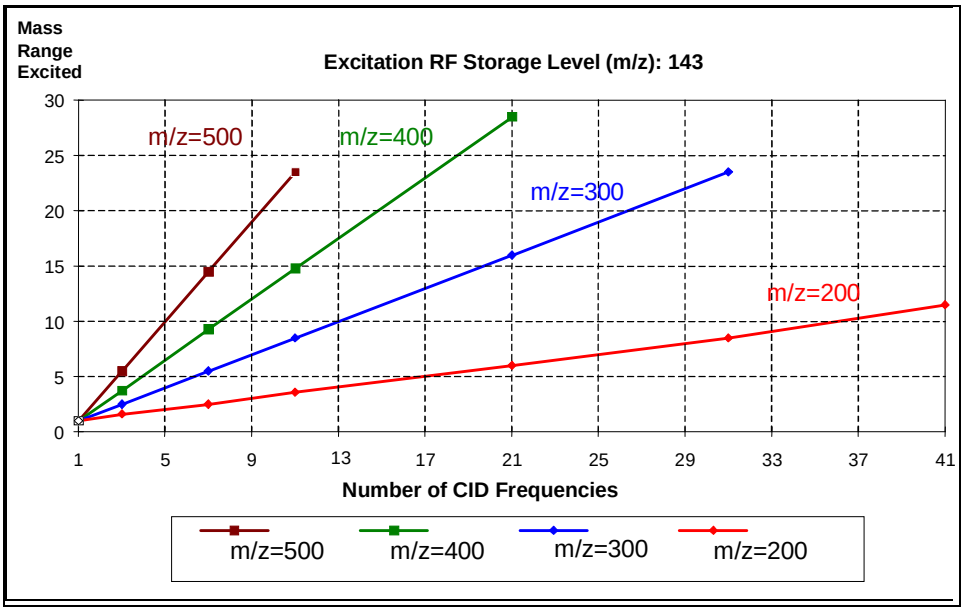
on the magnitude of the RF field, modulating the RF field amplitude modulates the resonant frequency of the ion. Modulating this frequency causes the frequency of the ion to match periodically that of the applied supplemental dipole field. Thus, the energy coupled to ion motion is maximized, and the effects of shifts in the ion resonant frequency are minimized.

The resonant excitation method is selective for the mass range that is excited. It permits coupling of energy to the motion of an ion having a particular m/z value in a very controlled way. Consequently, at the correct excitation amplitude, the rate that the amplitude of the ion motion increases is balanced by the rate that energy is removed by collisions. The ion is not ejected and energy can be deposited as internal energy by increasing the number of collisions. Increasing the excitation time increases the number of collisions. Thus, it is possible to fragment precursor ions that require the breakage of multiple chemical bonds or that undergo significant rearrangements. Periodic modulation through ion resonance results in an averaging of the energy coupled into the ion motion and provides a reproducible product ion spectrum, even with changes in concentration.

An additional parameter is available with resonant excitation. Increasing the number of CID frequencies permits simultaneous excitation of ions within a range of masses. This may be useful when trying to excite an entire isotopic cluster at once. As the number of CID frequencies increases, the range of excited masses also increases. For a given number of CID frequencies, the range of excited masses increases with increasing ion mass, and decreases with increasing excitation RF storage level.



Mass range excited vs. number of CID frequencies for an excitation RF storage level of 48 m/z



Mass range excited vs. number of CID frequencies for an excitation RF storage level of 143 m/z

With a storage level of 48 m/z and with 9 CID frequencies, the mass range increases from 3 to 40 m/z when the precursor ion mass increases from 100 to 400 m/z. Given a precursor ion mass of 400 m/z and 9 CID frequencies, the mass range decreases from 40 m/z for a storage level of 48 m/z to a range of 12 m/z for a storage level of 143 m/z.

Product Ion Mass Scanning

Following formation of the product ions by collision-induced dissociation, an RF ramp is used with the supplemental waveforms to scan resonantly ions from the trap to the conversion dynode and electron multiplier. This process generates the product ion mass spectrum.

MS/MS on the 500-MS

The AMD scan type is a specialized scan function for Automated Methods Development. It allows a scan function to be built quickly if the only difference between microscans is the CID amplitude.

The method parameters can be separated according to each step.

The ionization parameters are identical to those for full scan. Some are on the ionization control tab, and others are included in the MS/MS parameters table.

Basic Parameters: These are available on the MS/MS tab of the method without clicking **Customize**.

Precursor Ion (m/z): The specified ion on which to perform MS/MS. Values range from 50 to 2000 Da. Fractional masses are allowed.

Polarity: Sets the polarity of the detected ions, either positive or negative.

Capillary Voltage (Volts): Effects the focusing of ions into the capillary as well as the energy imparted to them as they exit the capillary and enter the skimmer. Higher masses tend to require higher voltages. For a given ion, higher voltages usually cause more fragmentation. Values range from 0 to 300 volts, as appropriate for the selected polarity.

RF Loading (%): Changing this value optimizes the amount of energy that an ion acquires after injection into the ion trap. Lower values may minimize fragmentation for a given ion. Higher values provide a deeper potential well for trapping, which also imparts more energy to the ions to overcome the deeper potential well. Values range from 0 to 300%. Typical values are 50 to 150%. A value of 100% works well for wide scan ranges.

Isolation Window (m/z): As the parent ion is isolated in the ion trap, this parameter specifies how many masses are stored. For example, a window of three stores the precursor ion, an ion one Dalton higher in m/z and an ion one Dalton lower in m/z.

The values depend on the precursor ion selected. For low m/z ions, this total isolation window is from 1 to 3 Daltons. For high m/z ions, this total isolation window is from 1 to 14 Daltons.

Excitation Storage Level (m/z): Determines the q value for the ion during the dissociation step. The higher the q values the more energy is imparted to ions. This also indicates the lowest m/z product ion that can be stored and subsequently scanned in the MS/MS spectrum.

The value depends on the precursor ion m/z, for q values from 0.1 to 0.6. The software automatically enters a value based on the precursor ion m/z.

Excitation Amplitude: Determines how much energy is imparted to the ion during the CID process. Higher values give the ion more kinetic energy, making collisions with the helium damping gas more energetic. If amplitude is set too high, the ions are ejected during the excitation step and no product ions are formed. The optimum value depends on the stability of the ion and the excitation storage level.

Allowable values are 0 to 59.79 volts for resonant excitation waveforms and 0 to 119.58 volts for non-resonant excitation waveforms.

The software automatically enters an approximate value based on the precursor ion m/z and the excitation storage level.

Product Ion Start Mass (m/z): Specifies the lowest mass that appears in the MS/MS spectrum.

Values range from the excitation storage level to the precursor ion minus seven Daltons.

Product Ion End Mass (m/z): This value specifies the highest mass that appears in the MS/MS spectrum. Values range from the product ion start mass to 2000 Daltons.

More Ranges: Click the more ranges check box to specify non-contiguous mass ranges for the MS/MS spectrum.

Possible ranges are the start and end masses described above, with the additional constraint, segments must have a minimum of 10 Daltons between them.

The following parameters, displayed when customize is clicked, allow for control over a wide range of parameters.

Isolation Waveform: Check this to turn on an isolation waveform to use as the ion trap is loaded. Use this to reduce the intensity of low mass matrix ions, which reduce the storage capacity for the precursor ion. NOTE: this waveform often reduces the intensity of the precursor ion.

Low Offset (m/z): This is specified in Daltons and is used to reduce the size of the isolation window on the low m/z side.
Values range from -0.7 to + 0.7 Da. The usual value is 0 Da.

High Offset (m/z): This is specified in Daltons and is used to reduce the size of the isolation window on the high m/z side.

The values range from -1.0 to + 1.0 Da. The usual value is 0 Da.

High Mass Ejection Factor (%): The high mass ejection factor specifies the relative amplitude of the isolation waveform that removes ions at a higher m/z than the precursor ion.

The values range from 0 to 200%. Typically, this value is 100% unless the precursor ion prematurely dissociated in the isolation step.

Waveform Type: The CID excitation waveform is either resonant or non-resonant. For a description of these waveforms, please see the section of this manual entitled MSMS - Tandem Mass spectrometry.

Excitation Time (msec): This is the time the excitation waveform is applied. Generally, longer times require smaller excitation amplitudes, so optimize these two parameters together.

Values range from 0 to 650 msec. The algorithm uses the default value of 10 msec when the excitation storage level and the excitation amplitude are automatically computed.

Modulate RF: If using resonant excitation, checking this causes the q value at which the ion is stored to be repeatedly ramped in a small region around the specified value. This effect slightly changes the resonant frequency of the ion and ensures that the ion is in resonance with the applied CID frequency under all conditions. The default is for this to be checked.

Number of Frequencies: If using resonant excitation, this parameter specifies the total number of frequencies applied. By increasing the number of frequencies used for excitation, the user may excite a broad range of ions, which is useful for dissociating all of the ions in a halogen cluster. *NOTE: The excitation amplitude used with this feature must be increased proportionally with the number of frequencies used.* Values are odd numbers of frequencies from 1 to 119.

CID Frequency Offset (kHz): If using resonant excitation, this parameter allows the applied CID frequency to be different from the calculated frequency by the amount a user specifies. This is useful for advanced users who operate with the RF modulation turned off. Values range from -10 to +10 hertz.

Injecting Samples

This section describes instrument setup, operation, and data acquisition and uses **System Control** and **Method Editor**. Acquiring data may involve these steps:

- Create or modify an existing method that includes sections for your 500-MS, HPLC modules, and AutoSampler. It may also include sections for data processing
- Set up and plumb the valve for manual injection
- Set up a SampleList that may include standards and unknown analytes
- Set up RecalcLists (where appropriate) for analysis of data
- Prepare and load the samples into the AutoSampler

The following sections are a general overview of these steps and the steps required for optimization of the 500-MS system.

Overview

When making a single injection with the 500-MS LC/MS system, use the **Inject Single Sample** button. It defines the sample and method that it used in the analysis. When you programming multiple injections through automation, use a SampleList to name each sample and specify the injection.

Making injections with your 500-MS system requires that these three steps be completed prior to starting your analyses:

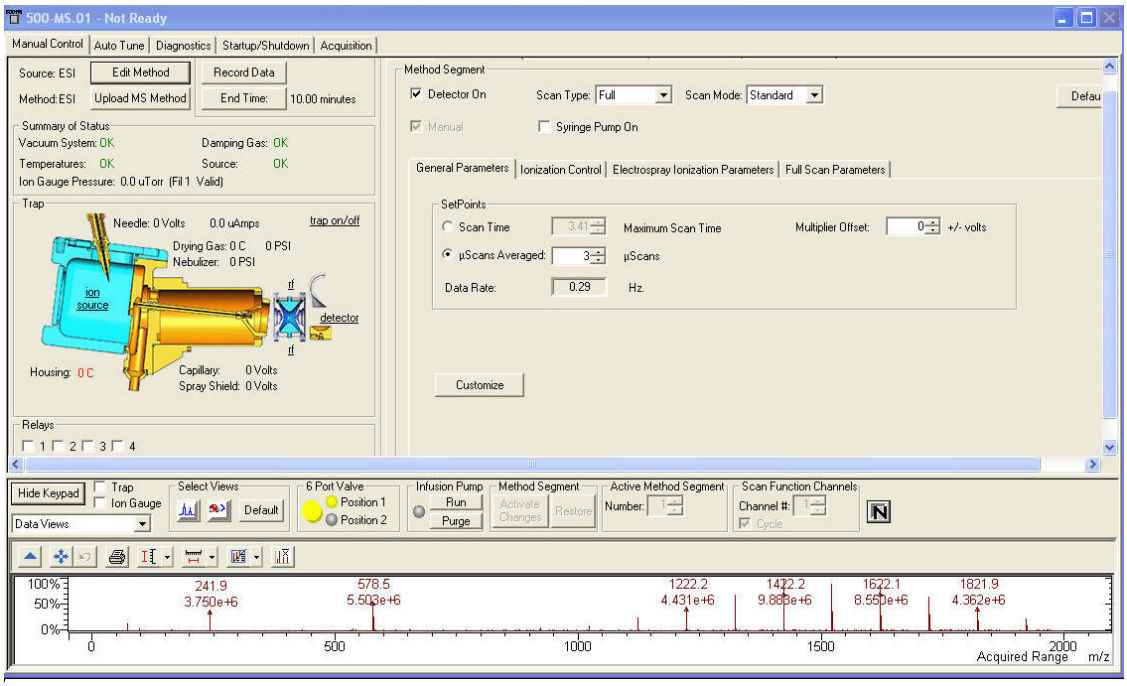
1. The 500-MS and the necessary chromatography modules were powered on and have reached their operational setpoints.
2. The sample(s) are prepared and ready to be analyzed.
3. A method with the desired analytical conditions exists.

Step 1 involves turning on the various components if they are not already on, making sure there is enough solvent in HPLC solvent reservoirs, paper in printers, the MS is pumped down, tuned, and any set temperatures have been reached and are stable.

Step 2 requires that the samples be in solution, diluted and any other sample specific preparations necessary to analyzing the sample are completed.

Step 3 involves editing the conditions specified in the method for the devices you are using. Editing the various sections of the method in the **Method Editor**, save the edits, and activate the method in **System Control**.

To begin, click the **System Control** button , if it is not open.



ESI Startup / Shutdown

Startup

Click the **Trap** check box in the 500-MS View to turn on the gases and heaters. If the method requires the infusion pump, set the drying gas to 10-15 psi (see the following sections). If the method requires flow injection, or uses the LC pumps, set the drying gas to 20 psi or greater. Allow a few minutes for the temperature and gas flows to stabilize before beginning analysis.

Shutdown

Turn off the flow from the LC pumps and allow the LC pump pressure to drop and stabilize so the flow into the ESI stops. Click the **Trap** check box to turn off the gasses and heaters. The Analyzer turned off automatically when you turn off the trap.

Pump Infusion and Manual Injection

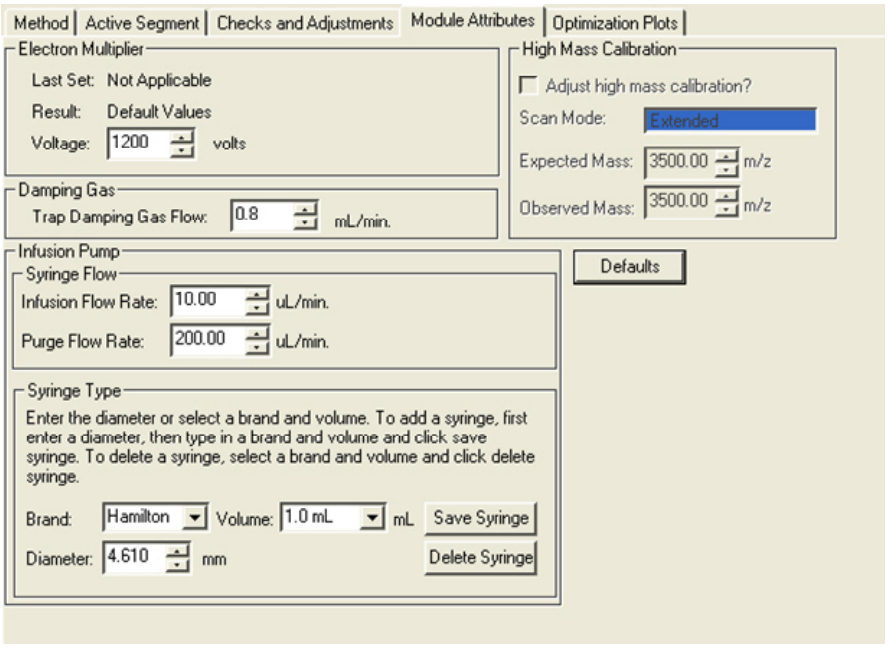
Infusion with Syringe Pump

Use the motor driven syringe pump on the 500-MS to infuse samples into the source for purposes of tuning and optimizing electronic and physical settings. Typical infusion rates are 10-20 µL/min.

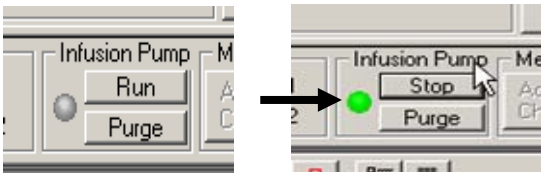
Connect the syringe to the luer end of the UCP-659 adapter and connect the other end to the PEEK™ nut on the PEEK line going into the MS.

Load a syringe with the sample at a suitable concentration (approx. 1 ppm) in an appropriate solvent. Set the syringe type in the 500-MS software by clicking the **Module Attributes** tab and selecting the syringe type and size in the appropriate boxes. The following example uses a 1.0 mL Hamilton syringe with a flow rate of 10 µL/min.

If syringe pump control is specified in the method, the flow rate is disabled.

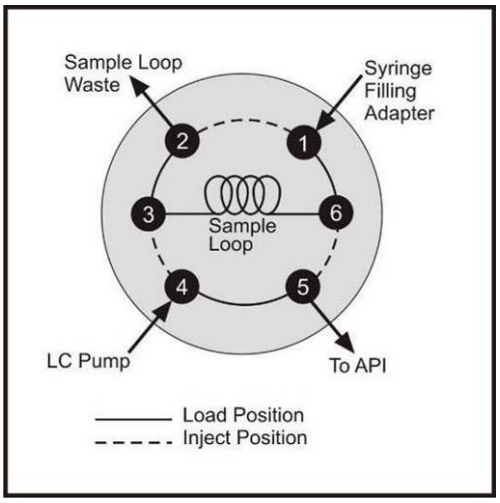

Method
Active Segment
Checks and Adjustments
Module Attributes
Optimization Plots
Electron Multiplier
Last Set: Not Applicable
Result: Default Values
Voltage: 1200 volts
High Mass Calibration
Adjust high mass calibration?
Scan Mode: Extended
Expected Mass: 3500.00 m/z
Observed Mass: 3500.00 m/z
Damping Gas
Trap Damping Gas Flow: 0.8 mL/min.
Infusion Pump
Defaults
Syringe Flow
Infusion Flow Rate: 10.00 uL/min.
Purge Flow Rate: 200.00 uL/min.
Syringe Type
Enter the diameter or select a brand and volume. To add a syringe, first enter a diameter, then type in a brand and volume and click save syringe. To delete a syringe, select a brand and volume and click delete syringe.
Brand: Hamilton
Volume: 1.0 mL
mL
Save Syringe
Diameter: 4.610 mm
Delete Syringe

Click **Run** under infusion pump and check that the green indicator comes on.

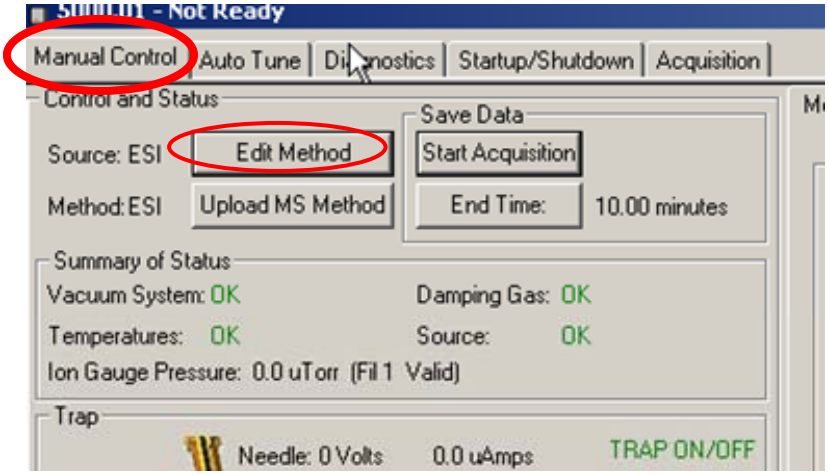


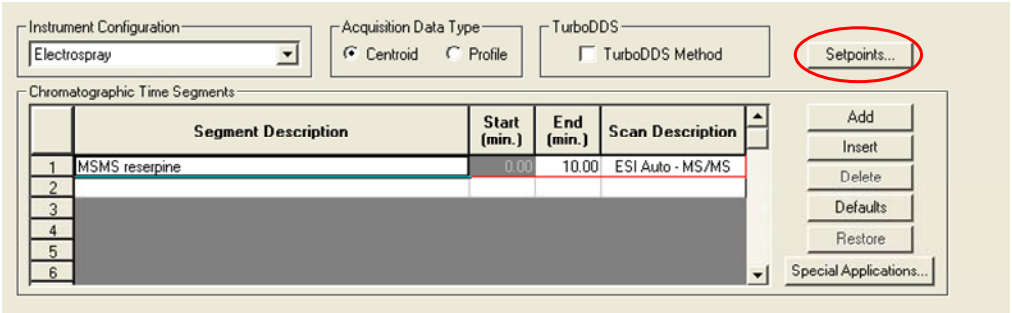
Manual Injection

To make a manual injection, plumb the switching valve as follows.



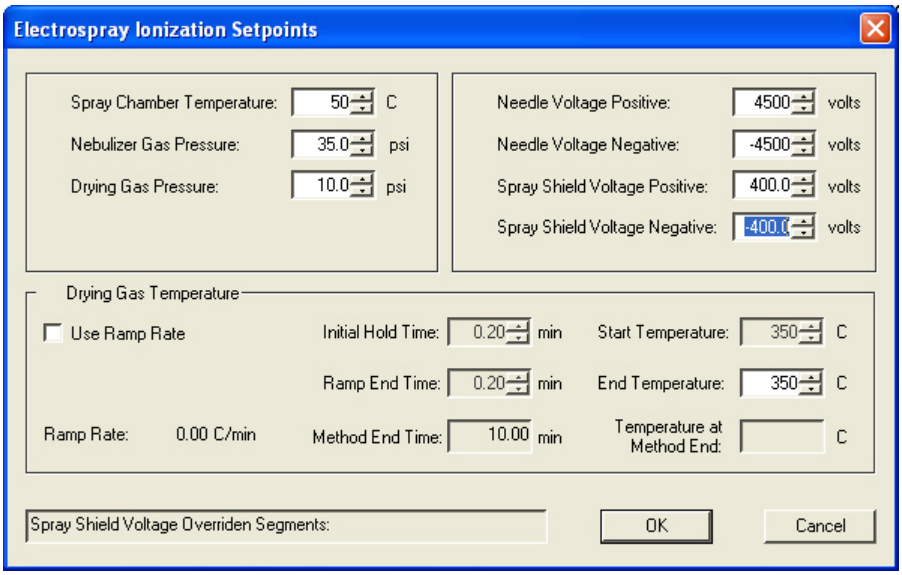
In the **Manual Control** tab, click **Edit Method** then **Setpoints**.



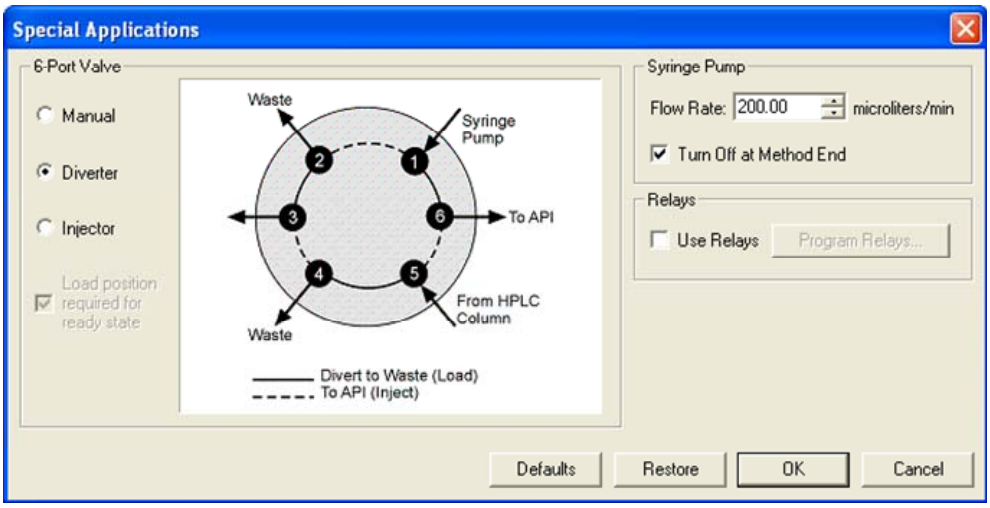


To set ESI Parameters:

1. Select a value between 10 and 15 psi for the **Drying Gas Pressure.**
   a. Check that **Use Ramp Rate** is not selected.
   b. Enter the **End Temperature** for the drying gas.
   c. Click **OK** to save the changes.

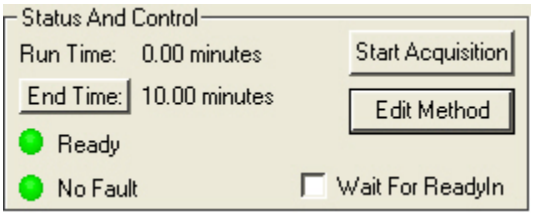

Electrospray Ionization Setpoints
Spray Chamber Temperature: 50 C
Nebulizer Gas Pressure: 35.0 psi
Drying Gas Pressure: 10.0 psi
Needle Voltage Positive: 4500 volts
Needle Voltage Negative: -4500 volts
Spray Shield Voltage Positive: 400.0 volts
Spray Shield Voltage Negative: -400.0 volts
Drying Gas Temperature
Use Ramp Rate
Initial Hold Time: 0.20 min
Start Temperature: 350 C
Ramp End Time: 0.20 min
End Temperature: 350 C
Ramp Rate: 0.00 C/min
Method End Time: 10.00 min
Temperature at Method End: C
Spray Shield Voltage Overriden Segments:
OK
Cancel

Click **Special Applications** in the **Method** tab and click **Diverter**.



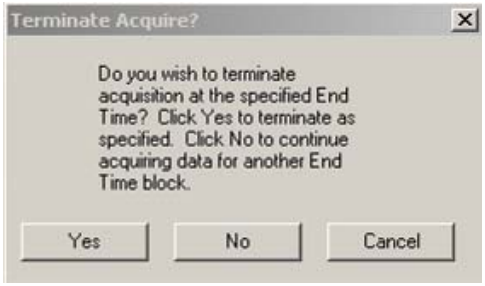
Save the method and click **Activate Changes**.

Click the **Acquisition** tab and set the end time for data collection by clicking **End Time** and entering a value. Click **Start Acquisition** and the 500-MS will begin to acquire data with the method specified.



Check that the valve is in the load position; overfill the 5 µL loop of the switching valve by a factor of 2 or 3. Push the yellow button on the front of the instrument to inject the sample. Allow at least 1 minute after a peak eludes before making another injection.

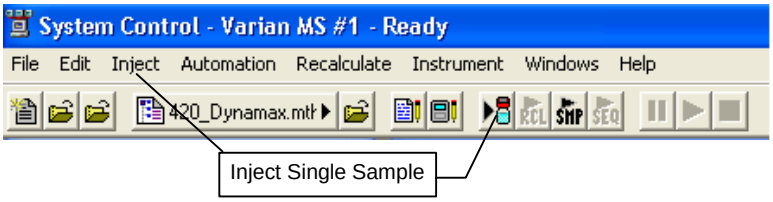
As the acquisition runs its course, several seconds before the end of acquisition, the 500-MS will ask if additional time is required for the current acquisition. Follow the instructions in the dialog to either add more time or end the acquisition.


Terminate Acquire?
Do you wish to terminate acquisition at the specified End Time? Click Yes to terminate as specified. Click No to continue acquiring data for another End Time block.
Yes
No
Cancel

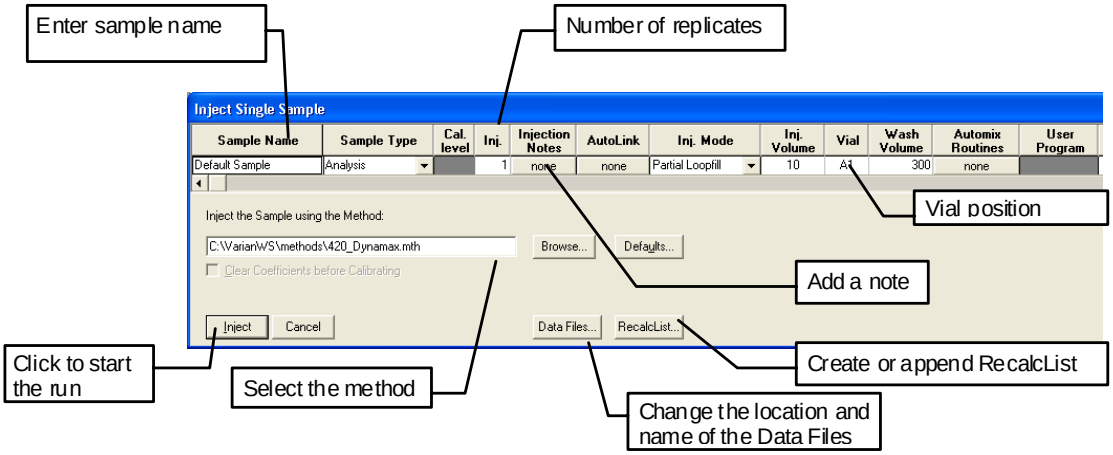
Injecting Single Sample

This example assumes you have an autosampler such as the Varian 300/400 series or HTS PAL configured with your 500-MS LC/MS. Refer to the appropriate section of this manual or the hardware manual to properly configure and plumb the autosampler.

To perform one injection, click the **Inject Single Sample** icon on the **System Control** menu bar. Alternatively, you can display the **Inject Single Sample** dialog by selecting it from the **Inject** menu.

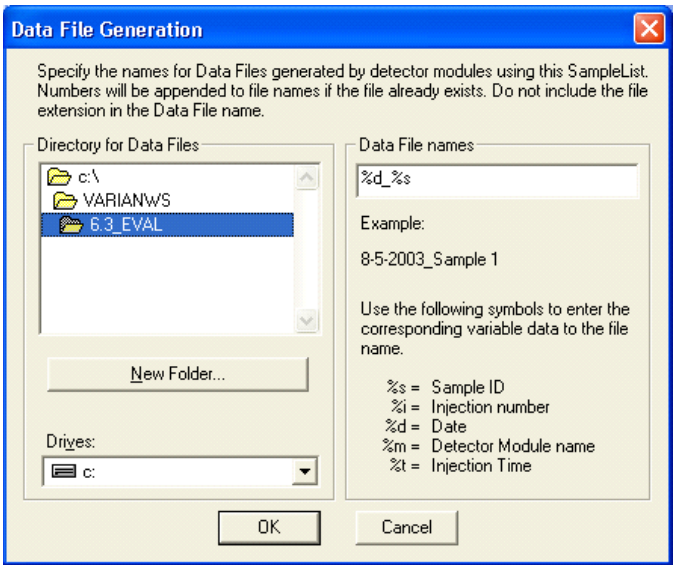

System Control - Varian MS #1 - Ready
File Edit Inject Automation Recalculate Instrument Windows Help
420_Dynamax.mth
Inject Single Sample

Click **Inject Single Sample** to display the following dialog box. The fields in the table are specific to the device configured in the instrument.



Specifying the Data File Name and Path

Data File names can be up to 255 characters long. Sample ID, injection date, module name, and injection number can be embedded in the file name making the Data File name correlate with each sample injection. When you click the **Data Files**, the Data Files Generation dialog box opens. This dialog box allows you to select the path and the filename specification for the data file.


Data File Generation
Specify the names for Data Files generated by detector modules using this SampleList. Numbers will be appended to file names if the file already exists. Do not include the file extension in the Data File name.
Directory for Data Files
c:\
VARIANWS
6.3_EVAL
New Folder...
Drives:
c:
Data File names
%d_%s
Example:
8-5-2003_Sample 1
Use the following symbols to enter the corresponding variable data to the file name.
%s = Sample ID
%i = Injection number
%d = Date
%m = Detector Module name
%t = Injection Time
OK
Cancel

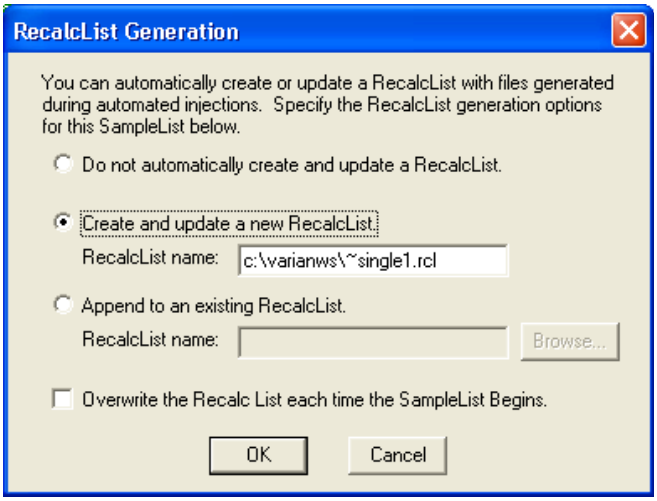
The left side of the **Data File Generation** dialog box allows you to select the drive letter and subdirectory (path) where the data files are to be stored. The default directory is the data subdirectory of your VarianWS directory.

The right side of this dialog box allows you to create a filename “specification”. You can combine text entry with the “%” variable symbols shown to specify filenames that contain sample injection specific information. An example of the filename is dynamically updated as you type in the filename specification. This makes it easy to see how a data file created with this filename specification would appear.

Specifying a RecalcList

The files created by the execution of the SampleList can be stored in a RecalcList, which keeps track of the names of the created files, and other parameters. The RecalcList can be reprocessed later (for example, to reprint all the reports or to process all the data with another method).

You can choose to create a new RecalcList, append to an existing RecalcList, or neither create nor update a RecalcList. To select the RecalcList option that you want, click the appropriate RecalcList button. The RecalcList Generation dialog box opens.

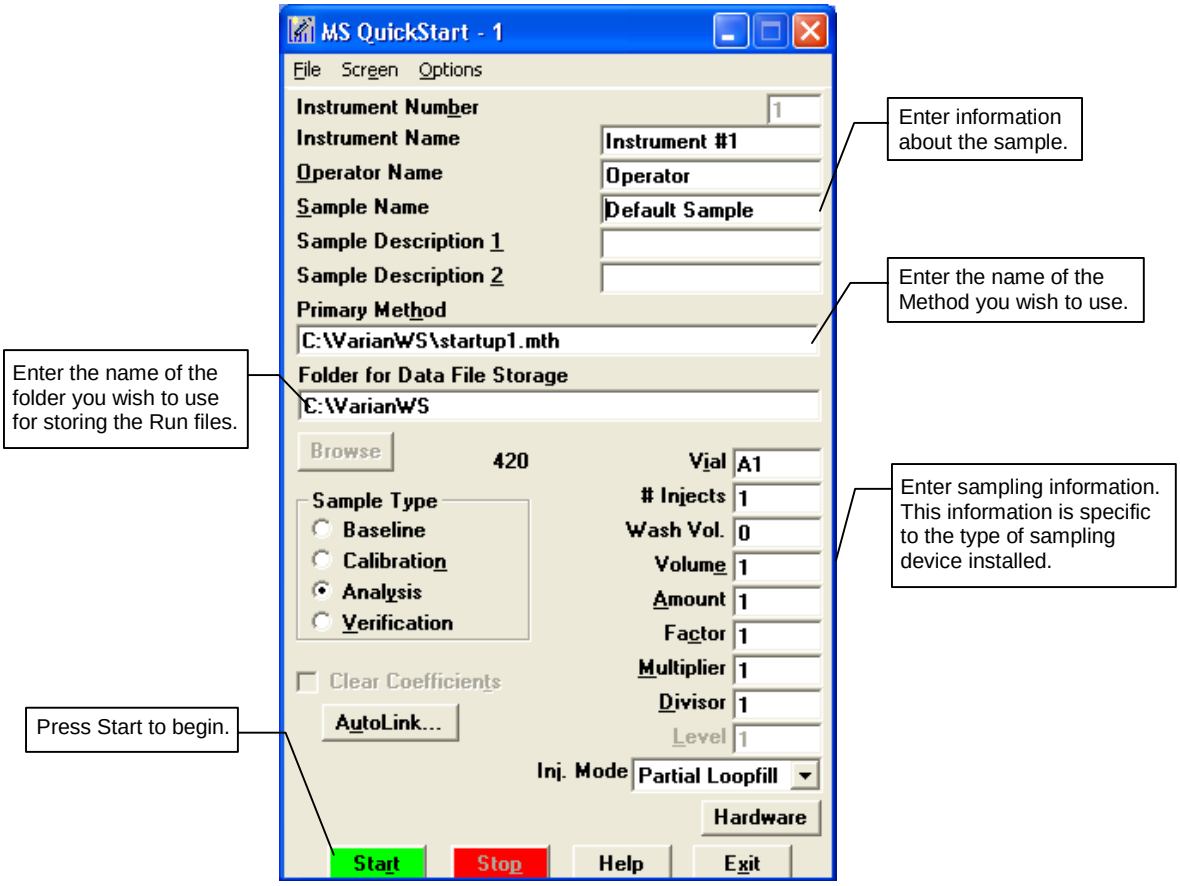

RecalcList Generation
You can automatically create or update a RecalcList with files generated during automated injections. Specify the RecalcList generation options for this SampleList below.
Do not automatically create and update a RecalcList.
Create and update a new RecalcList.
RecalcList name:
c:\varianws\~single1.rcl
Append to an existing RecalcList.
RecalcList name:
Browse...
Overwrite the Recalc List each time the SampleList Begins.
OK
Cancel

If you choose to create a new RecalcList, the automatically generated RecalcList does not overwrite an existing RecalcList unless you also specify “Overwrite the RecalcList each time the SampleList begins”. If a RecalcList with the same filename exists, the newly created RecalcList has a number appended to its filename to make it unique and to prevent the older RecalcList from being overwritten.

Using QuickStart

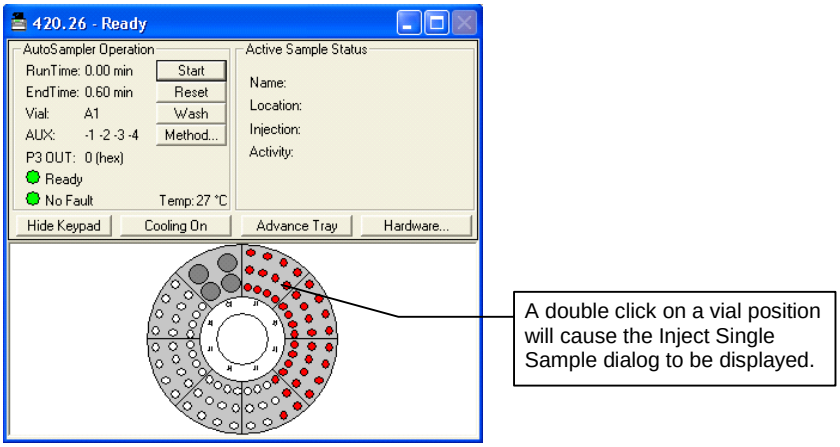
QuickStart is a fast way to inject a single sample without having to use System Control directly. QuickStart can be customized and is ideal when setting up instruments for routine use. Refer to the online help for further details.

QuickStart starts System Control and waits until all modules are in a ready state. When ready, the QuickStart window opens.



Selecting a Vial from the AutoSampler Display

Each supported AutoSampler has a graphic representation of the carousel System Control. You may do an individual injection of any vial by double-clicking the appropriate vial in the display. The Inject Single Sample dialog appears and, after you enter the information, the injection is done.



Using Inject Single Sample for Priority Samples

Use either the graphical vial display or the Inject Single Sample dialog to set up a priority sample in conjunction with automated analyses.

To inject a priority sample:

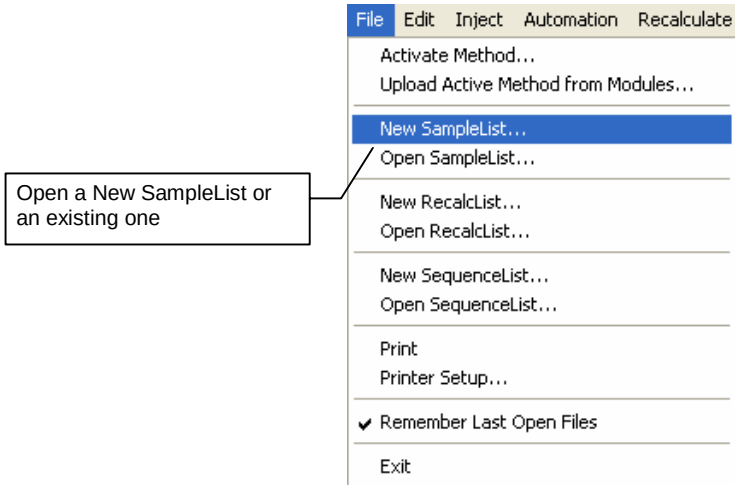
1. While automation is running, click **Suspend** on the current active SampleList. Automation is suspended after the current run is completed.
2. Double-click the vial position where you placed your high priority sample or choose inject single sample as described above and perform your injection.
3. After completion of the priority run, you can resume the SampleList that you were running by clicking **Resume** on the bottom of the SampleList.

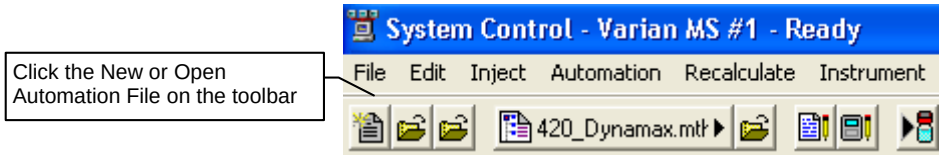
Injecting Multiple Samples

Your instrument should be configured and you should have a basic method for data acquisition. If this has not been done, please refer to the appropriate sections of this manual for further instructions.

Using a SampleList in System Control

You can inject multiple samples from System Control by using the SampleList.

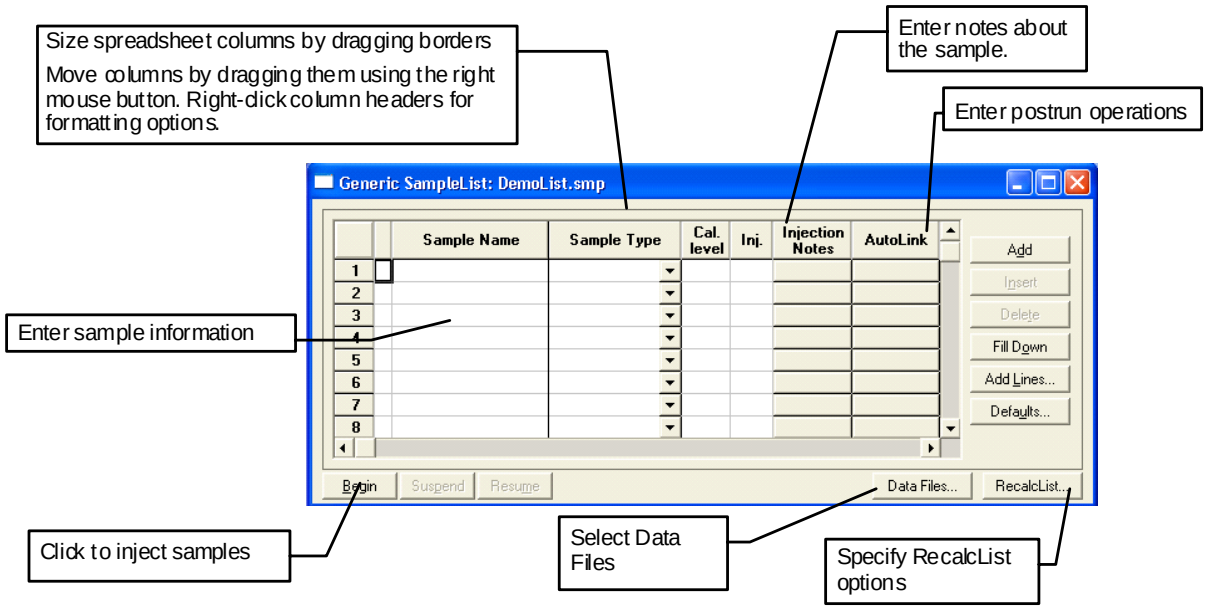




The SampleList window for the open SampleList opens. It contains fields that are specific to the sampling device configured in the instrument.

Generic SampleList

If there is no sampling device configured in the Instrument, a generic SampleList is used which does not contain columns for selecting vial positions, sampling options or hardware configuration options.

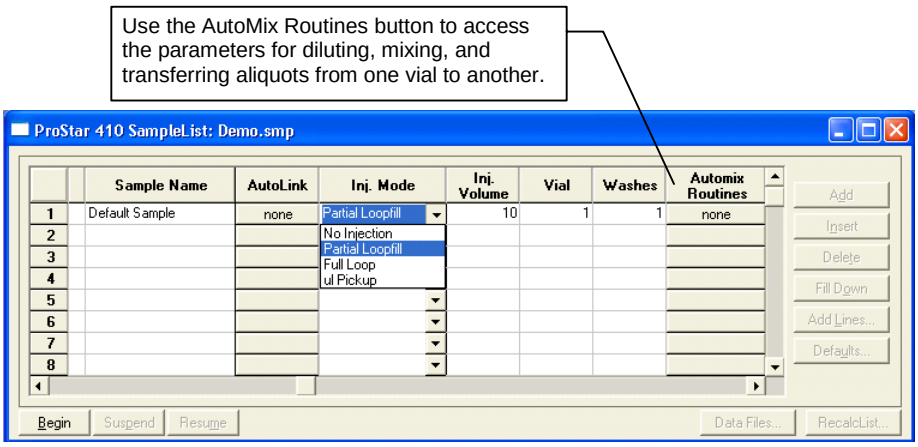


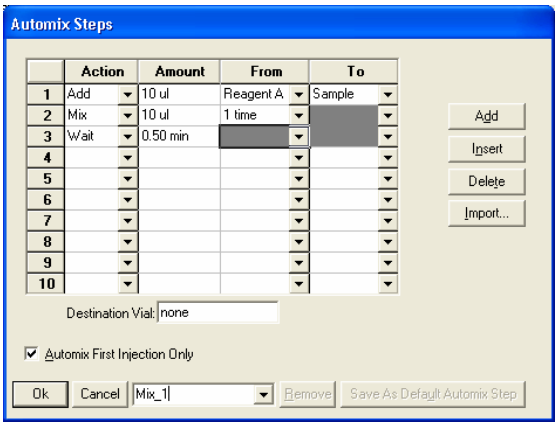
When the table is scrolled to the right, the *Sample Name* column does not scroll so you can easily tell for which sample you are entering additional parameters for commonly used data handling parameters, the amount for single internal standard calculations, the unidentified peak factor, a multiplier, and a divisor, can be entered directly into this table. If you have more complex requirements, such as multiple internal standards or multiple detectors requiring different entries, click the button in MultiChannel MultiStandard column.

Each AutoSampler is supported by its unique SampleList. A SampleList may have more than one AutoSampler section and may be used for different AutoSamplers. The information entered for one AutoSampler section of the SampleList is not displayed or used in other AutoSampler sections. SampleLists are the method by which an AutoSampler program is created since AutoSamplers operate on a per sample basis as opposed to the time resolved method for pumps and detectors.

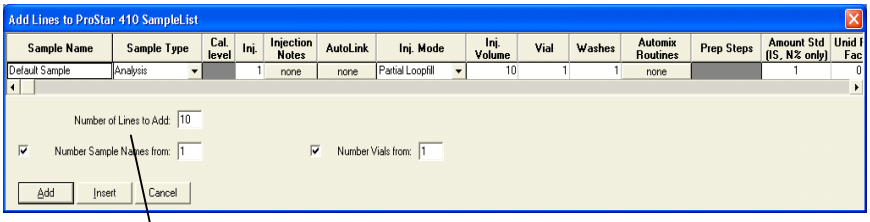
ProStar 410 SampleList

In addition to the Generic SampleList features that were described earlier, the PS-410 supports a variety of other features such as different Injection Modes, Vial specification, and AutoMix functions.



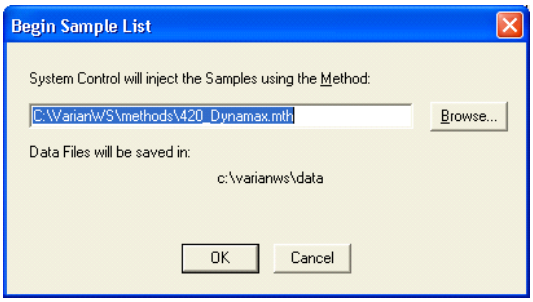


To add several similar lines to the SampleList, click **Add Lines**. You can then enter the common information in the dialog box.



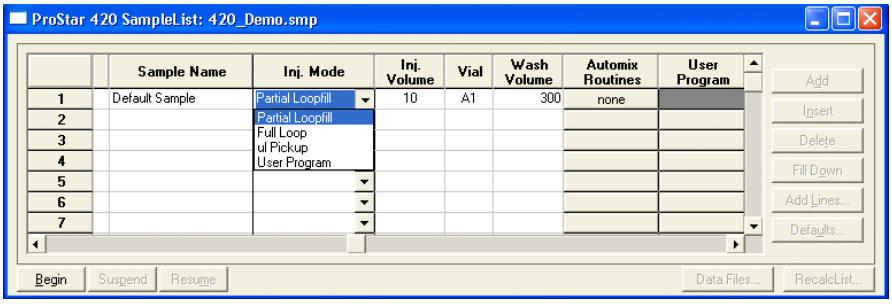
For sequentially numbered Sample Names, enter the starting number and the number of entries to add to the SampleList. The Sample Names will have these numbers appended to them.

When you click **Begin** on the SampleList, you are prompted for the Method to use. You can use the active Method that is pre-selected, or you can Browse for another Method. After you click **OK**, the run will begin.



ProStar 420 and 430 SampleList

The PS-420 and PS-430 SampleLists are similar to the generic System Control SampleList, with additional columns to support operations specific to each AutoSampler. Use the Injection Mode column to select one of the three methods of injection; Partial Loopfill, Full Loop, and µL Pickup. The User Program can also be selected from this column. Several columns of the SampleList are disabled when the User Program is selected.



For the PS-430, use the Plate column to specify the plate location of the well or sample of interest. You can enter a plate number between 1 and 7. This column is disabled unless the PS-430 is equipped with the Plate Feeder option.

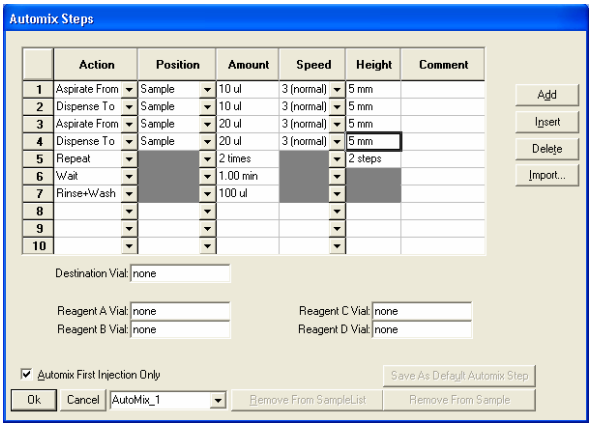
The PS-430 Well/Vial column specifies the well or vial from which you want to aspirate the sample for injection. The location is specified as a combination of row letter and column number. The available rows and column numbers depend on the type of plate that is configured in the AutoSampler Hardware. You can enter A1 through F8 for the 48 standard vials, A1 through H12 for the 96 well plates and A1 through P24 for the 384 well plates. You can use the Well/Vial column to randomly access any well or vial; for example, you can periodically recalibrate using a single standard at a fixed location.

For the PS-420, the Vial column specifies the vial of interest. The location is specified as a combination of Tray type letter and Vial number. The available Tray types and Vial numbers depend on the Tray Segments configured in the PS-420 Hardware. You can use the Vial column to access randomly any vial; for example, you can periodically recalibrate using a single standard at a fixed location.

The Wash Volume column is used to specify a wash volume in the range of 300 to 9999 µL, or specify 0 for no wash.

The Automix Routines column allows you to define a specific automixing program to be done before injecting the sample. When performing an Automix routine, you can program the AutoSampler to aspirate a specified amount of liquid from a

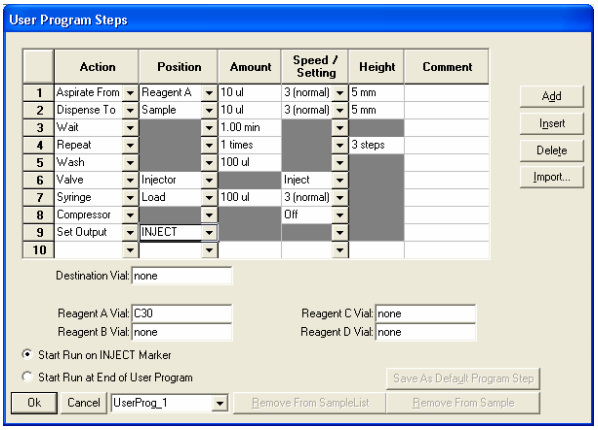
Well, Vial, or a Reagent Vial, and then transfer that liquid to the sample Well or Vial from which an injection is to be made. You can mix the sample by aspirating and dispensing specified amounts of the mixture, and you can program the AutoSampler to wait for a reaction to take place. In the Automix Steps dialog, you can select Action, Position, Amount, Speed of the syringe, and Height of the needle. The following is an example of an automix routine.



Up to 240 steps can be programmed in one automix routine. After entering all the steps required for handling the sample, enter a name for this Automix Program in the box next to **Cancel** and click **OK**. The program is saved in the SampleList, and can be exported to other SampleLists. Alternatively, Automix Programs from other SampleLists can be imported to the active SampleList using **Import**.

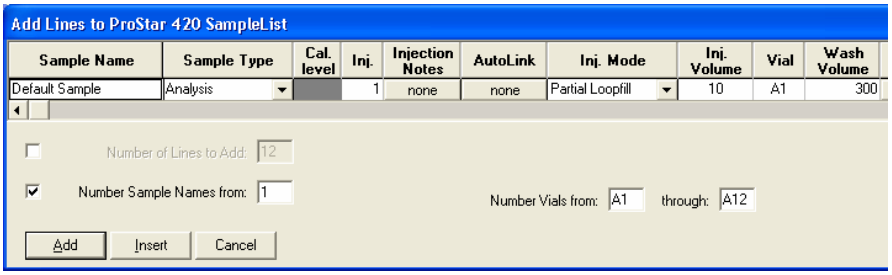
The User Program column is enabled if you select the User Program in the Injection Mode column for this sample. The User Program programs all possible actions required for a sample handling and injection that can be executed by the AutoSampler in separate steps.

When the User Program is selected as the Injection Mode, the following SampleList columns are disabled; Injection Volume, Wash Volume and Automix Routines. The User Program steps are used instead of the parameters in the Method. If you use the User Program, the 10-mL syringe must be selected in the Hardware Configuration. The following figure shows a list of the User Program Actions that can be specified. For each specified action, there could be several available options such as Position, Amounts, Speed Setting, and Height.



You can select when to Start the Run, either on INJECT Marker (step 9 in the above example), or at the End of User Program. After entering all the steps required for handling the sample, enter a name for this User Program in the box next to **Cancel** and click **OK** (UserProg_1 in the above example). This User Program is then saved in the SampleList, and can be exported to other SampleLists. Alternatively, User Programs from other SampleLists can be imported to the active SampleList using **Import**.

When you are ready to build a PS-420 SampleList or a PS-430 SampleList, you can use **Add** at the right side of the SampleList to enter sample lines efficiently.



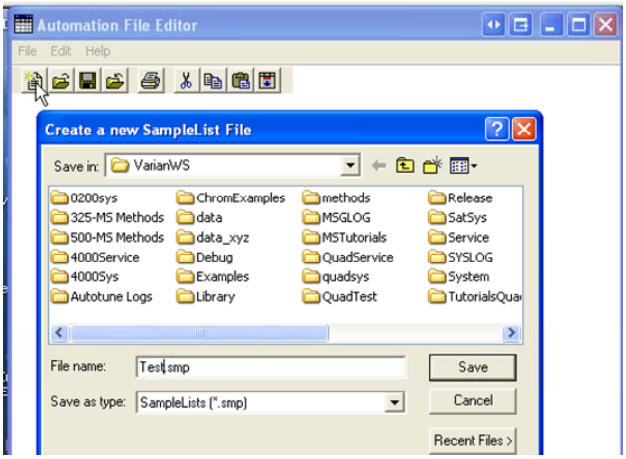
The **Add** dialog allows you to enter common information and select the number of entries to be added sequentially to the SampleList. With the PS-420, you can specify the first and last vials to be added to the SampleList with the requirement that the first and last vial numbers start with the same Tray Type letter, (A, B, C, or D).

With the PS-430, you can specify the first and last Row and Column to be added to the SampleList. You can also select to have the entries added by Rows or Columns, which determines if the plate is processed from left to right by Rows or from top to bottom by Columns.

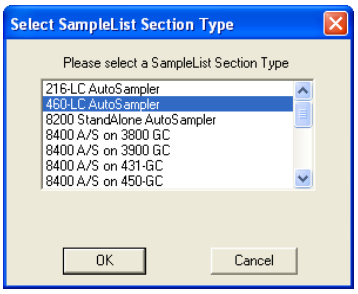
460-LC SampleList

To create a 460-LC SampleList, do the following:

1. Click the Edit Automation Files button to open the Automation File Editor.
2. Click the New Automation File button and click new SampleList.
3. Enter a file name and click **Save**.

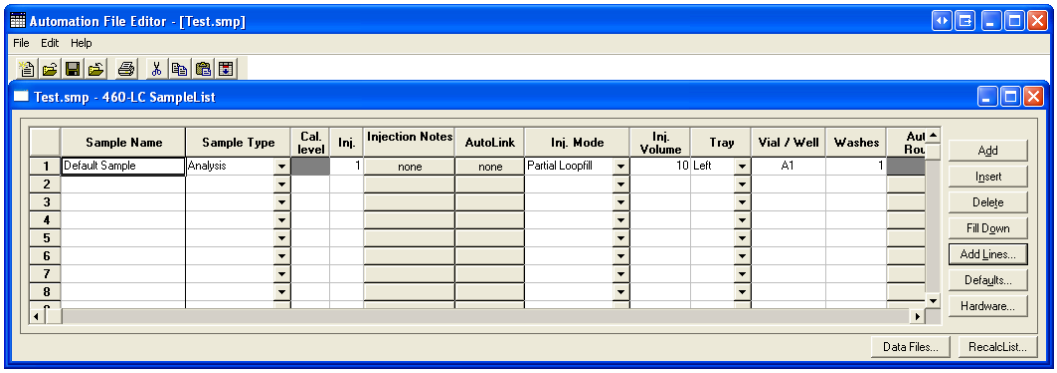


4. Select the 460-LC AutoSampler and click OK.

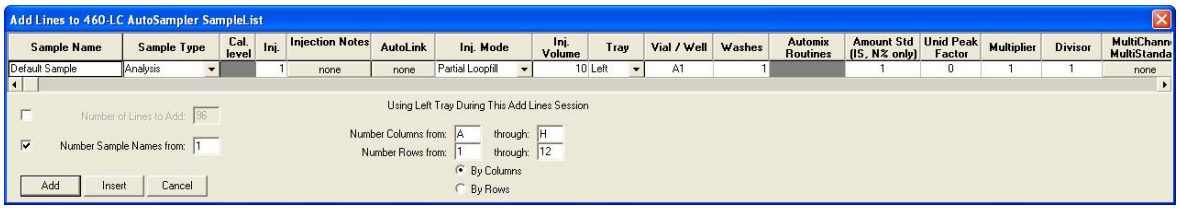


5. An empty SampleList with your file name opens

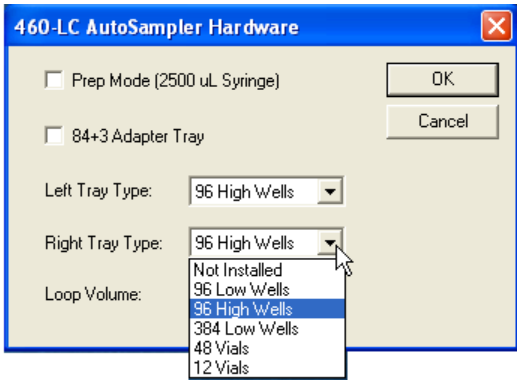
- Click **Add** to add one sample.



- Click **Add Lines** to add several samples or a plate.

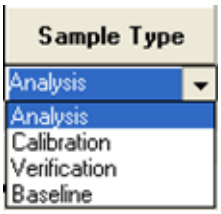


- Click **Hardware** to specify the tray type for each position, sample loop volume and indicate if the Syringe for Prep Mode is installed.



Sample Name: Enter the “root” name for all samples.

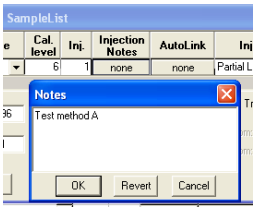
Sample Type: Select **Analysis**, **Calibration**, **Verification**, or **Baseline** from the menu.

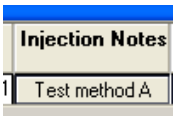


Cal. Level: This numerical field is used with Calibration and Verification Sample Types.

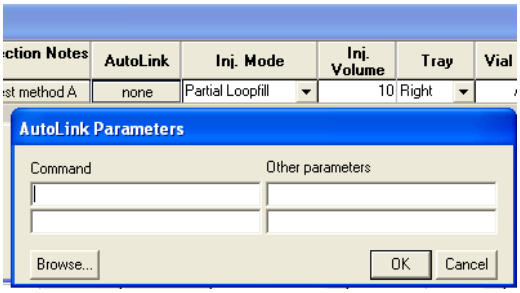
Inj. Enter the number of injections in this numerical field.

Injection Notes: Enter a note about the sample or the injections in this text field.





AutoLink: Add a Command or Other parameters for post run event.



Injection Mode: Select **No Injection**, **Partial Loopfill**, **Full Loop**, or **µL Pickup** from the menu.

Inj. Volume: The 460-LC comes with a 100 µL sample loop. Injections can be made with 1 µL increments.

Tray: Create a sample list for either the **Left** or **Right** tray.

Vial/Well: Enter a combination of a row letter and a column number. The available rows and column numbers depend on the type of plate configured.

- 96 well plate: A1 through H12
- 384 well plate: A1 through P24

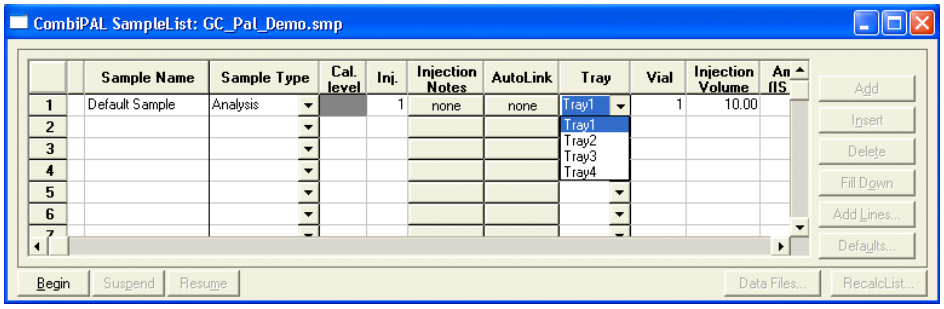
Wash Volume: Enter 1 to 9 washes.

Automix Routines: Use these routines only with the 84+3 Adapter Tray.

Combi PAL SampleList

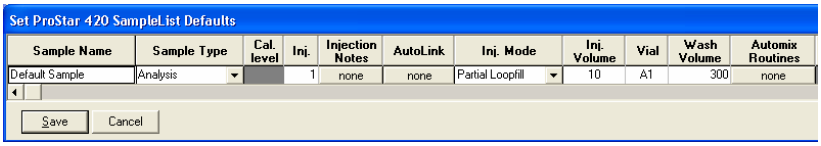
The Combi PAL has settings for Tray selection, Vial position, and Injection Volume in addition to the features of the Generic SampleList.

All other sampling parameters are specified in the Combi PAL section of the Method.



Changing Default SampleList Entries

When you add a new row to a SampleList, default values are entered. To change the default values, click **Defaults** in the open SampleList window. The following dialog box opens. Enter the desired default values and click **Save**.



Note that neither volume nor rack and vial number is displayed in this screen even if an AutoSampler is present.

Saving SampleLists for Later Use

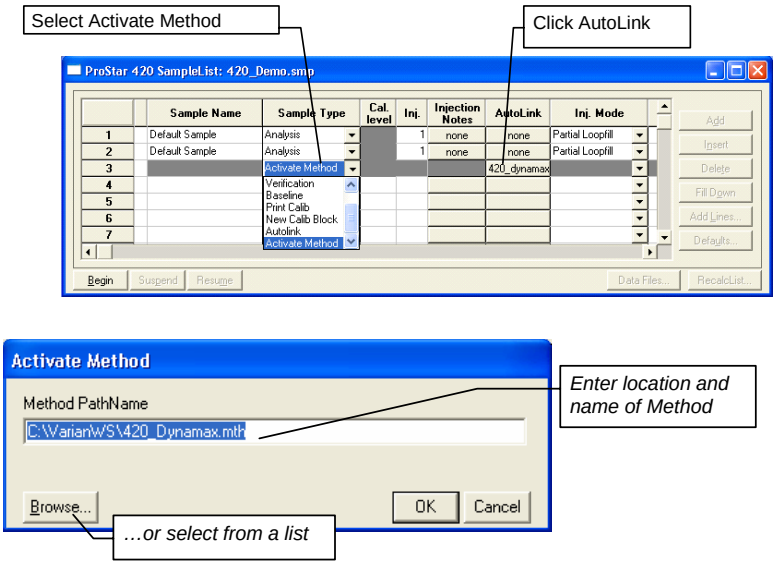
When you make changes to the open SampleList, they are automatically saved to the SampleList file and are used for the automated runs that are in progress. If you want to edit a SampleList other than the open SampleList, use the offline Automation File Editor application described in the next section.

Using More Than One Method for Injections

The MS Workstation allows you to perform automated injections using more than one Method. There are two ways in which this can be accomplished. The first is by changing the active Method from within the SampleList. The second is by using a SequenceList.

Changing the Method in the SampleList

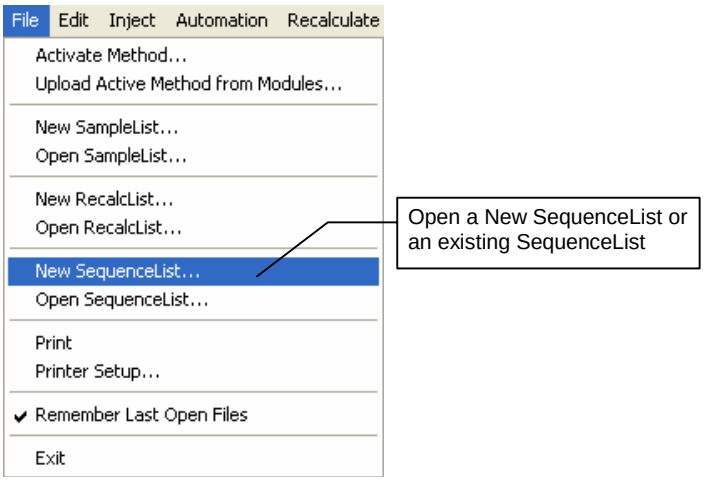
You may change the Method by activating a Method in a SampleList row.

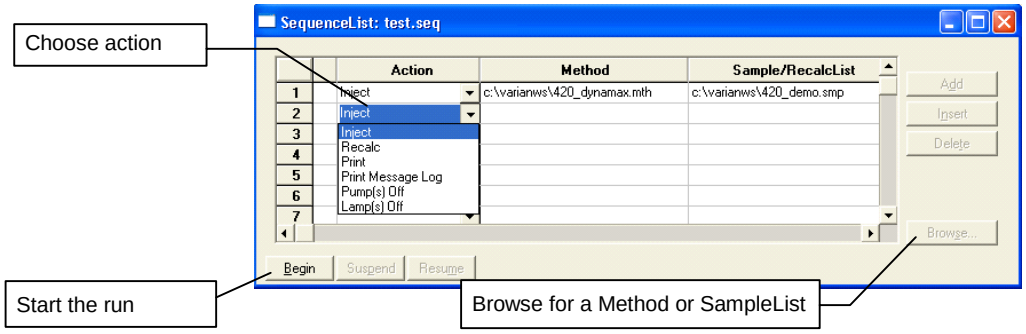


Specify any number of Methods in the SampleList.

Using the SequenceList Window

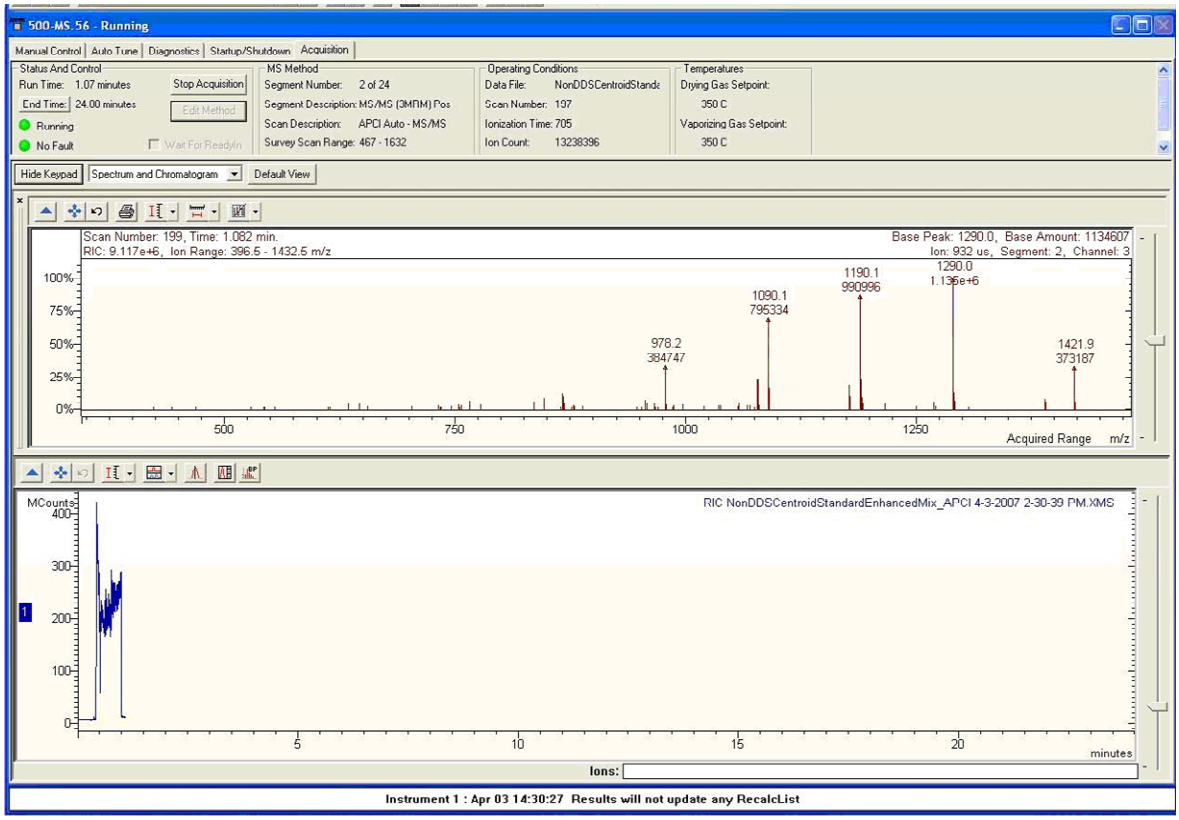
The SequenceList window allows you to specify multiple Methods and SampleLists to be processed during automation.





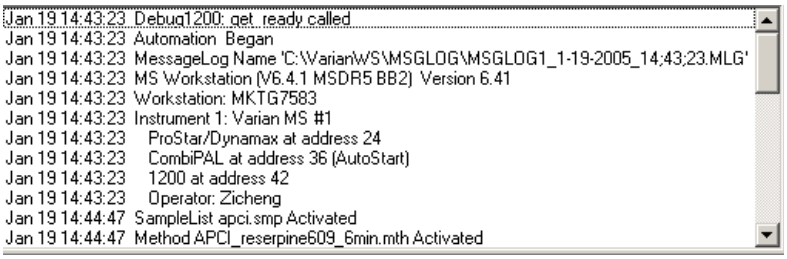
Monitoring Run Status

After making an injection, monitor the status in the instrument window.



The module status is shown in the status and control windows and on the Toolbar.

Automation actions and errors are logged in the Message Log; the most recent line of this log is at the bottom of the screen. When you double-click the status bar at the bottom of the instrument window, the entire Message Log window opens.

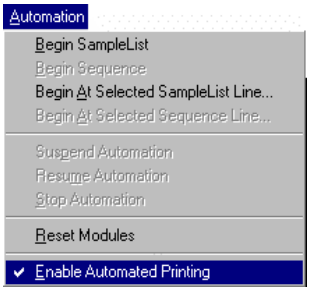

Jan 19 14:43:23 Debug1200: get_ready called
Jan 19 14:43:23 Automation Began
Jan 19 14:43:23 MessageLog Name 'C:\VarianWS\MSGLOG\MSGLOG1_1-19-2005_14;43;23.MLG'
Jan 19 14:43:23 MS Workstation (V6.4.1 MSDR5 BB2) Version 6.41
Jan 19 14:43:23 Workstation: MKTG7583
Jan 19 14:43:23 Instrument 1: Varian MS #1
Jan 19 14:43:23 ProStar/Dynamax at address 24
Jan 19 14:43:23 CombiPAL at address 36 (AutoStart)
Jan 19 14:43:23 1200 at address 42
Jan 19 14:43:23 Operator: Zicheng
Jan 19 14:44:47 SampleList apci.smp Activated
Jan 19 14:44:47 Method APCI_reserpine609_6min.mth Activated

Message Log entries are stamped with the date and time they occurred.

Automated MS Report Generation

After adding Report sections to your Method, automated reports can be generated from System Control after each injection, after Recalculations, or by using any of the Print actions in the Sequence window.

You can disable automated Report printing during the course of an automated sequence of injections or Recalculations. Disable automated printing from the Automation menu in the Instrument window.



When this item is not checked, report printing is disabled. ASCII file generation is still performed if specified in the Report Method section.

Disabling automated printing is like to disconnecting the printer, data is acquired but no reports are printed.

Stopping an Acquisition

During automation and while the LC/MS system is running you can suspend or stop data acquisition as follows:

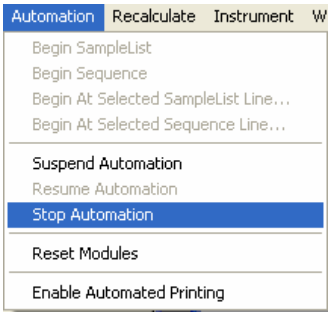
Suspend Automation: Suspends execution of automation after the current run has been completed.

Stop Automation: Stops the current run, resets all modules simultaneously, and suspends automation.

Resume Automation: Resumes execution of automation after it was suspended.

Reset Modules: Stops the current run, and resets all modules simultaneously. Automation proceeds to the next injection after all the modules go to the Ready state.

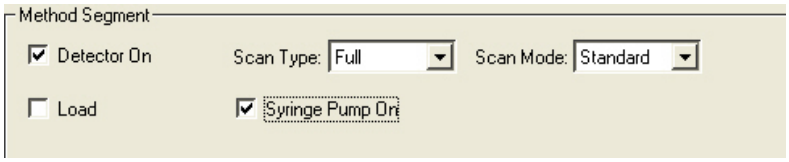
Select from the following in the Automation menu in System Control.



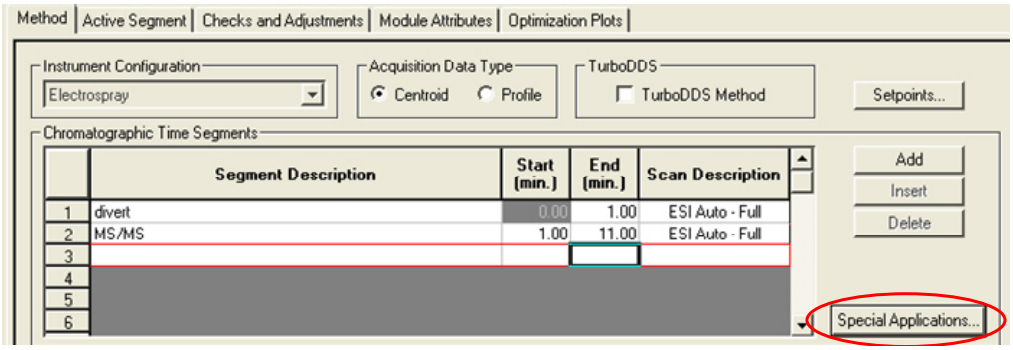
Setting a Collect/Divert Delay

Use the diverter valve to divert the injection front away from the API source. Frequently, the solvent front contains un-retained, non-volatile salts from the sample. These salts deposited in the source, build up over time and the source must be cleaned regularly. Diverting the solvent front reduces maintenance on the Source and API Interface. The delay parameter is used to switch the diverter valve to waste.

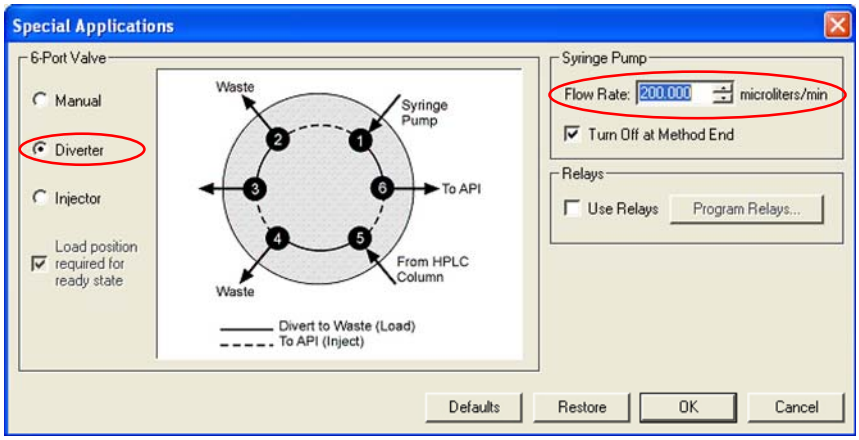
In the Method Segment section, enable **Syringe Pump On**.



Click **Edit Method** and add a segment to the 500-MS Method Segment section. Give the segment an appropriate name (in this case “divert”) and set the time for the end of the segment (1 min, in this example). Note, you can also have a divert segment at the end of the method to divert column effluent during a column washing step. In this case, the divert segment is inserted after the MS/MS segment shown in the following example.



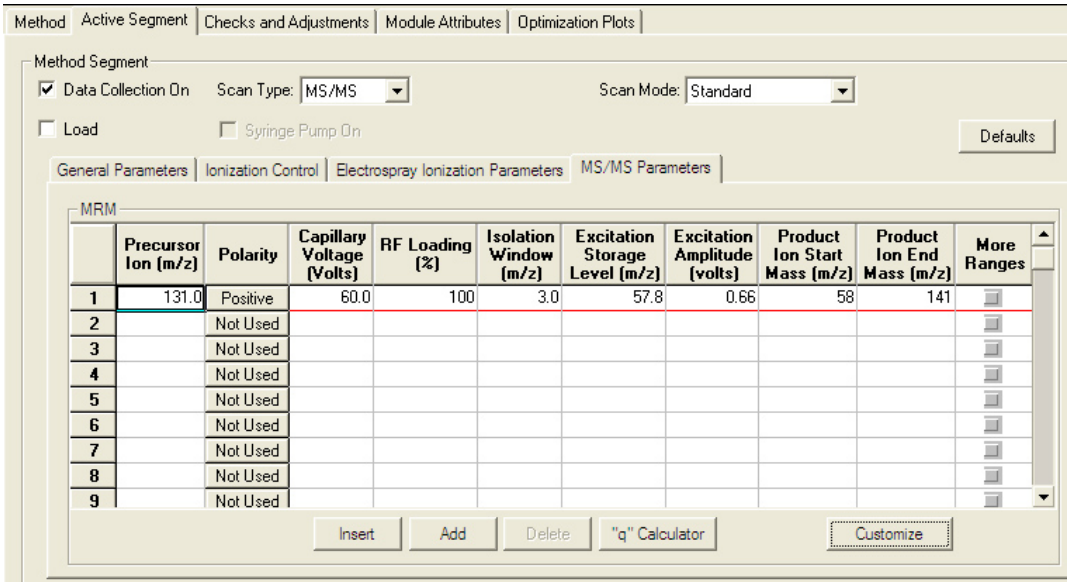
Set the valve type to **Divert** in the **Special Applications** window. Enter the **Flow Rate** of the Syringe Pump to make up for the lack of flow from the LC pumps during diverting. Plumb the 500-MS valve with the following configuration. In the diverter segment, check that the syringe pump is enabled.

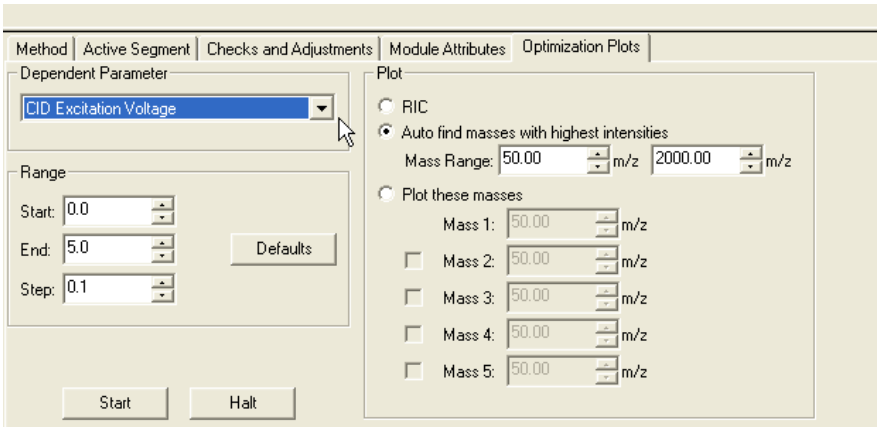


MS/MS Optimization

Use the 500-MS Optimization Plots View and the built-in syringe pump to help your MS/MS development process. Begin by infusing a sample in an appropriate solvent into the 500-MS, making sure your drying gas pressure and temperature are set to the desired levels. After you click **Start**, the Optimization Plot screen automatically appears adjacent to the Spectrum Plot screen. You can click and drag the **Bar** to undock the Plot, move, and resize it.

Click the **Optimization Plots** tab and select **CID Excitation Voltage**. If you know the product ion masses, click the **Plot these Masses** button, and enter the desired masses in the *Mass 1*…*Mass 2*… dialogue boxes. If the masses are unknown, click the **Auto find masses**… radio button and select a range to look for the desired product ions. Click **Start** and allow the 500-MS to generate an optimization plot of the desired mass or masses.





The optimization plots are displayed in the 500-MS screen.

Return to the method editor and enter the CID excitation voltage in the MS/MS Parameters in the method segment.

Automation File Editor

Overview

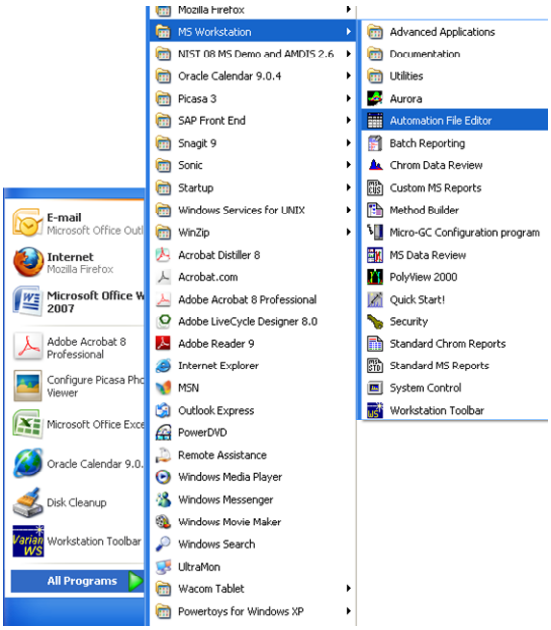
Use the Automation File Editor for creating and editing SampleLists, RecalcLists, and SequenceLists outside of the Varian MS Workstation System Control application. The off-line Automation File Editor allows access to these files without disrupting automated runs that may be occurring in System Control.

NOTE: SampleLists and Sequences that are active and running in System Control cannot be accessed simultaneously in the off-line Automation File Editor.

Accessing the Automation File Editor

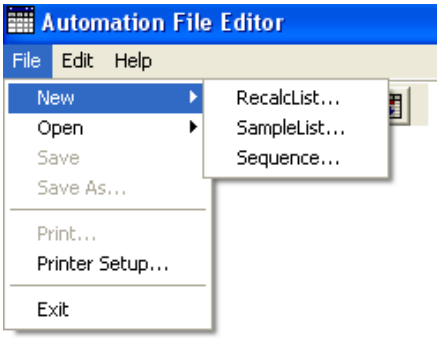
Click the **Automation File Editor** icon on the Workstation Toolbar to display the Automation File Editor.

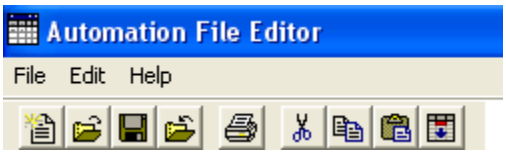
If the Workstation Toolbar is not already opened on your Varian MS Workstation, you can start the **Automation File Editor** from the Windows Start Menu.



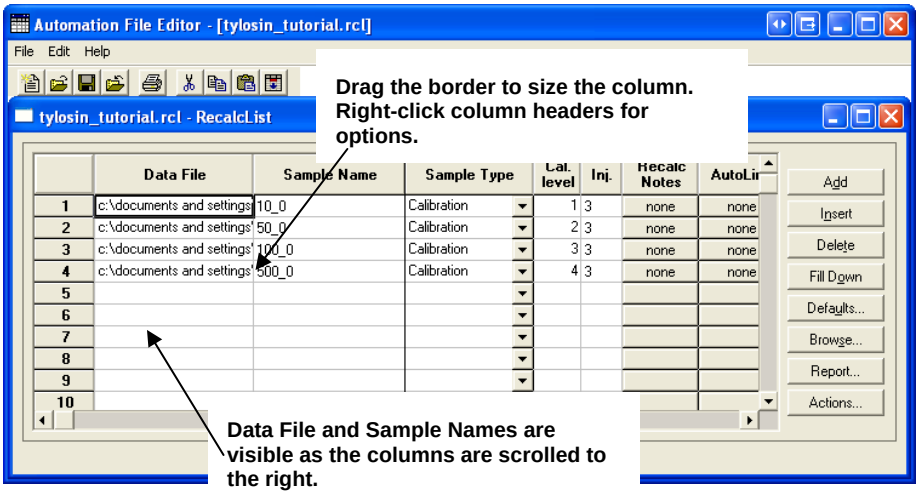
Creating or Editing a RecalcList

Either choose a New RecalcList or open an existing RecalcList from the File menu. Also, you can click **New** or **Open Automation File** on the toolbar.





The RecalcList window for the open RecalcList opens. It contains many of the fields of in the SampleList. While the SampleList contains AutoSampler and sample specific data handling information, the RecalcList contains the Data File name and data file specific data handling information. The fields that are common to both the RecalcList and the SampleList are described in the sections after the creation of the SampleList.

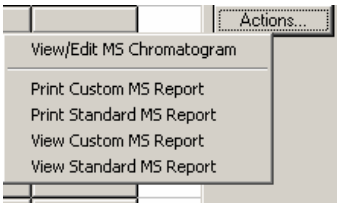


Recall Notes: Enter notes about the Recalculation.

AutoLink: Enter post calculation operations to be done.

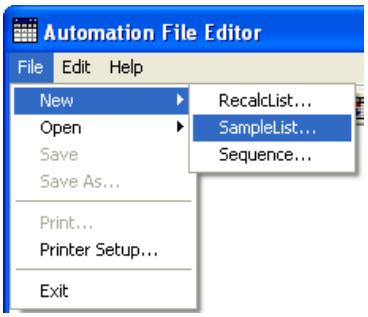
Report: Click **Report** to generate a report for the currently selected data file. Note that if a data file is not highlighted in the Data File column, no report is generated.

Actions: If a report was generated using the **Report** button, clicking **Actions** shows actions that may be done.

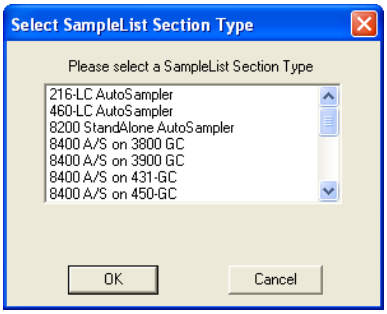


Creating or Editing a SampleList

Choose either a New SampleList or Open an existing SampleList from the File menu or by clicking **New** or **Open Automation File** on the toolbar.



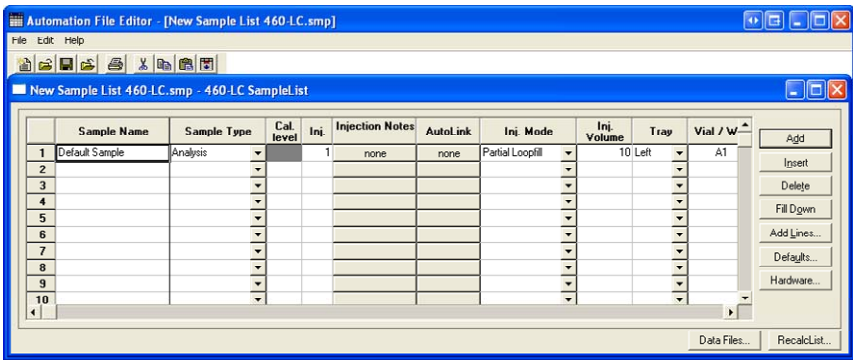
Select a list type that corresponds to the AutoSampler.



The SampleList window for the open SampleList type opens.

Click **Add** to add a default sample.

Click **Data Files** to select the location and name of Data Files.



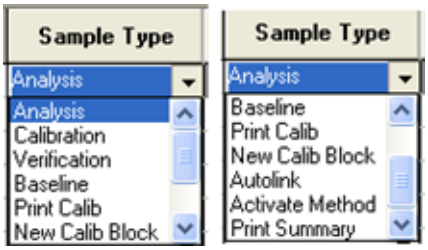
Drag the border of a spreadsheet to change the size. Right-click a column header to display formatting options. When the table is scrolled to the right, the Sample Name column does not scroll.

Defining the Fields

The top row of the SampleList window has fields for information sample. The various fields and buttons in this dialog are:

Sample Name: Enter the sample name. The final name of the file depends on settings accessed by clicking **Data Files**. If only '%s' is listed in the Data Files dialog the sample name will be as you entered it here.

Sample Type: Use the scroll bar to see all the options.



Cal. Level: If calibration is the sample type, you can designate up to ten calibration levels.

In: Enter the number of injections. You may make up to nine replicate injections at each calibration level.

Injection Notes: Enter notes.

AutoLink: Refer to the Specifying Data Handling Parameters section in this manual.

Vial	Injection Volume	Automix Routines	Amount Std (IS, N% only)	Unid Peak Factor	Multiplier	Divisor	MultiChannel MultiStandard
1	1.00	none	1	0	1	1	none

In the Vial, Injection Volume, and Injectors Used fields, enter information about the sample. The fields beginning with Amt. Std. are used only for data files acquired with standard LC detectors.

Add: Add a new line to the end of the list.

Insert: Insert a new line before the currently line.

Delete: Delete the selected line

Fill Down: When sections of columns or entire lines are highlighted, the information in the top highlighted row is repeated to the lowest selected row.

Add Lines: If you need to add several similar lines to the SampleList, click Add Lines. Enter the common information in the dialog box. Some fields in the **Add Lines** dialog vary from one AutoSampler to another. Only the fields that are related to the AutoSampler selected are displayed in this dialog.

For sequentially numbered Sample names, check the box and enter the starting number and the number of entries to add to the SampleList. The Sample Names will have these numbers appended to them.

Defaults: Change the default entries in the table, such as Injection Volume, Injectors Used, etc. After defining new defaults, click **Save** to activate the new defaults.

Data Files: Change the location and name of the Data Files.

RecalcList: Click to generate a RecalcList associated with single sample acquisitions.

Specifying the Data File Name and Path

Data File names can be up to 255 characters long. Sample ID, injection date, module name, and injection number can be embedded in the file name making the Data File name correlate with each sample injection. When you click **Data File**, the Data Files Generation dialog box opens. Select the path and the file name specification for the data file. The left side of the Data File Generation dialog box allows you to select the drive letter and subdirectory (path) to store the data files.

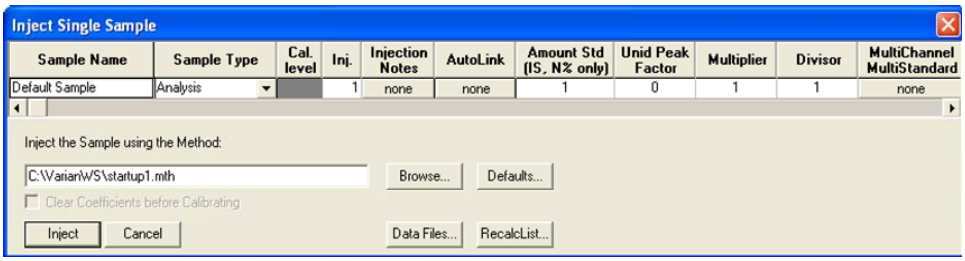
Error! Objects cannot be created from editing field codes.

Use the right side of this dialog box to create a file name specification. Combine text entry with the “%” variable symbols to specify file names that contain sample

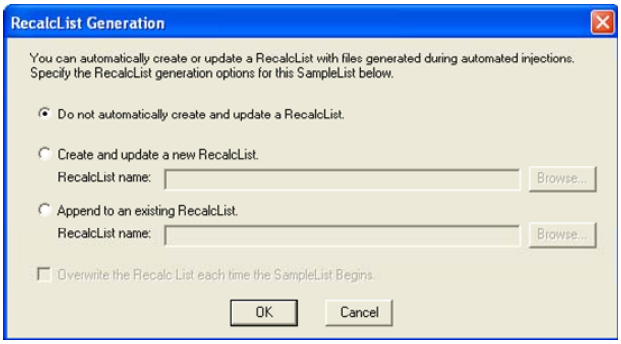
injection information. An example of the file name is updated as you enter the file name specification. This feature makes it easy to see how a Data File created with this file name appears.

Specifying a RecalcList

In **System Control**, click **Inject Single Sample** and **OK.**



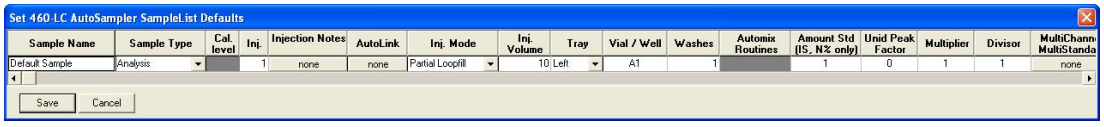
Create a new RecalcList, append an existing RecalcList, or neither create nor update a RecalcList. To select the RecalcList option that you want, click **RecalcList**. The RecalcList Generation dialog box opens.


RecalcList Generation
You can automatically create or update a RecalcList with files generated during automated injections.
Specify the RecalcList generation options for this SampleList below.
Do not automatically create and update a RecalcList.
Create and update a new RecalcList.
RecalcList name:
Browse...
Append to an existing RecalcList.
RecalcList name:
Browse...
Overwrite the Recalc List each time the SampleList Begins.
OK
Cancel

If you choose to create a new RecalcList, the automatically generated RecalcList does not overwrite an existing RecalcList. If a RecalcList with the same file name exists, the newly created RecalcList has a number appended to its file name to make it unique and to prevent the older RecalcList from being overwritten.

Changing Default SampleList Entries

When you add a new row to a SampleList, default values are used for each cell. To change the default values, click **Defaults** in the open SampleList window. The following opens. Enter the desired default values and click **Save**.



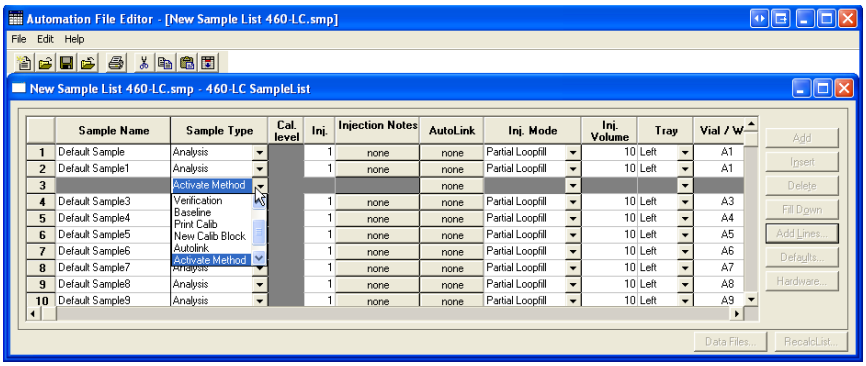
Using More Than One Method for Injections

You can do automated injections using more than one Method. There are two ways to do this.

- Change the active Method from within the SampleList.
- Use a Sequence.

Changing the Method in the SampleList

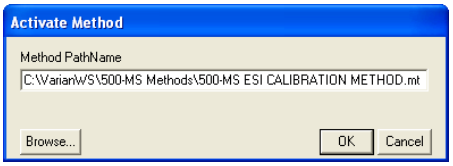
Change the Method used during injections by activating a Method in a SampleList row.



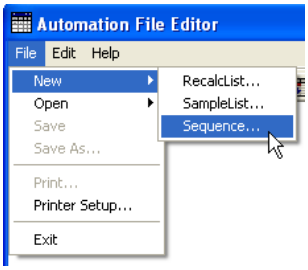
Select **Activate Method** from the Sample Type cell.

Click **AutoLink** in the desired row. The Activate Method window opens.

Enter the name of the Method or click **Browse** to select the Method. Specify any number of Methods in the SampleList.



Creating or Editing a Sequence



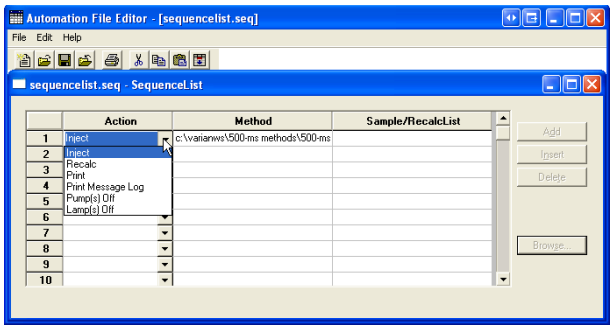
Either choose a New Sequence or Open an existing Sequence from the File menu.

The Sequence window for opens.

Click Add to add lines to the SequenceList. You may enter any number of Sequence lines.

Choose the action to be done in the selected step of the Sequence.

Enter the name of the Method or SampleList in the corresponding column, or use Browse to select the Method or SampleList from a list of files.



Security Administration

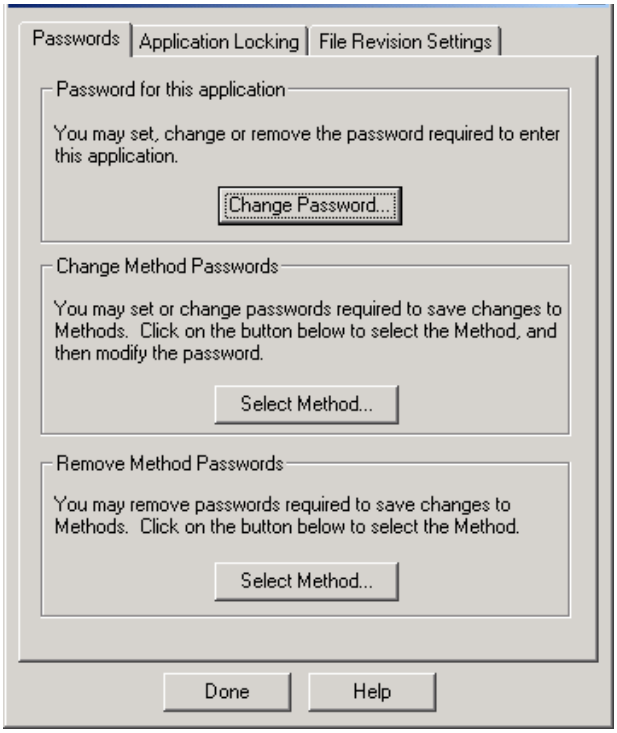
Overview

Use the Security Administration features to; set passwords, limit access to changing methods, require comments when methods changes are saved, and time and date stamp data files.

This window has three tabs.

- Passwords
- Application Locking
- File Revision Setting

Passwords


Passwords
Application Locking
File Revision Settings
Password for this application
You may set, change or remove the password required to enter this application.
Change Password...
Change Method Passwords
You may set or change passwords required to save changes to Methods. Click on the button below to select the Method, and then modify the password.
Select Method...
Remove Method Passwords
You may remove passwords required to save changes to Methods. Click on the button below to select the Method.
Select Method...
Done
Help

Password for this application: Specify a password for the Security Administration application.

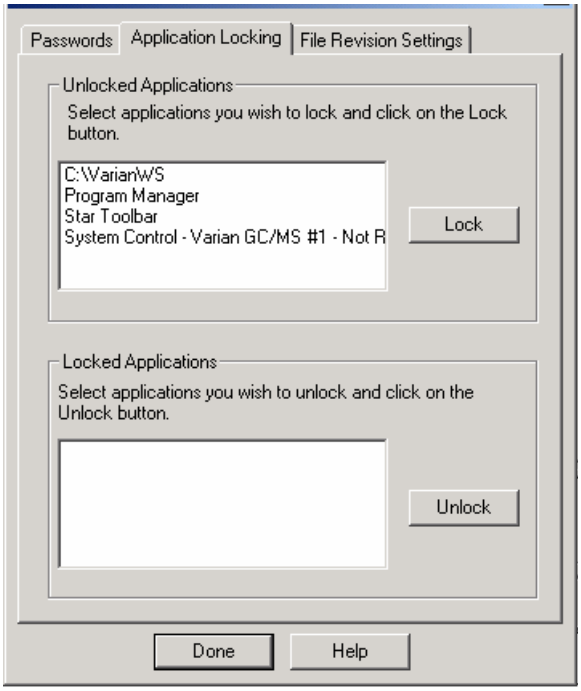
NOTE: Document the password in a secure location because it cannot be recovered.

Change Method Passwords. Method passwords can be added, changed, and removed from the Security Administration application. Click **Select Method** to browse for and select the desired Method. You are either prompted for a new password (if no previous password exists for the Method), for the old and new password (if the Method already contains a password).

NOTE: If creation of new passwords is disabled in the File Revision tab, you will not be able to add a new password to a Method that does not already contain one. Method passwords can also be added and changed in the Method Builder and Interactive Graphics applications.

Remove Method Passwords: Remove Method Passwords using Security Administration without entering the existing password. This is useful if the password for a Method has been lost. Click **Select Method**, select the desired Method, and the password is removed.

Application Locking

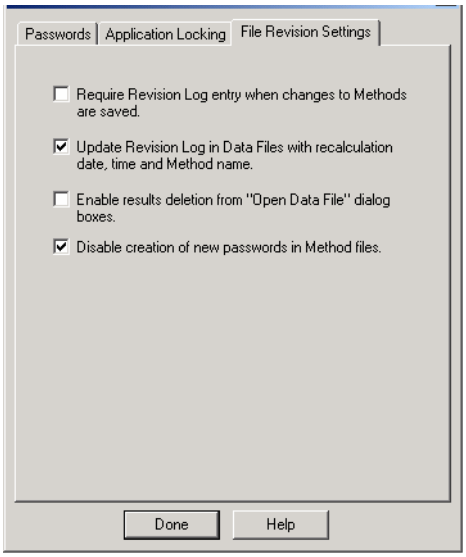


Unlocked Applications: Shows the top-level windows currently running that are not locked. Select any number of them, click **Lock** and they move to the Locked Applications list box and their windows are disabled (they will not respond to mouse or keyboard input).

Note that applications that are locked by the Security Administration application remain locked even after the Security Administration application is closed.

Locked Applications: Shows the top-level windows that are currently running and are locked. Select any number of them. Click Unlock, and they move to the Unlocked Applications list box and their windows are enabled (they respond to mouse or keyboard input).

File Revision Settings


Passwords
Application Locking
File Revision Settings
Require Revision Log entry when changes to Methods are saved.
Update Revision Log in Data Files with recalculation date, time and Method name.
Enable results deletion from "Open Data File" dialog boxes.
Disable creation of new passwords in Method files.
Done
Help

Require Revision Log entry when changes to Methods are saved: When checked, the user is prompted for a description of changes when a Method file is altered and saved. The Revision Log appears in the Notes portion of the **Open Method File** dialog box, is listed in the Method Builder application window when the Method is open, and is included in the Method printout under Revision History.

Update Revision Log in Data Files with Recalculation date, time, and Method name: When checked, Data Files are updated with a time stamp and Method name when they are recalculated (either from System Control or from Interactive Graphics). The Data File Revision Log can be included in printed reports.

Enable results deletion from "Open Data File" dialog boxes: When checked, a button appears in the **Open Data File** dialog box allowing results to be deleted from a specified channel of a Data File. Results deletion is logged in the Data File Revision Log. This option only affects standard LC results. LC/MS results are deleted.

Disable creation of new passwords in Method files: When checked, new passwords cannot be added to Methods. Methods already containing passwords will still require a password before changes are saved.

Configuring HPLC Modules

Overview

The first time that you use the Varian MS Workstation to collect chromatographic data, and generate results, you must configure the communications and modules in the System Control and Configuration Window. Set the communication parameters for each chromatograph modules, and drag the modules into the Varian MS Workstation instrument area. After this is done, the configuration is stored and you only need to reconfigure System Control when you change the physical hardware configuration of your chromatograph modules.

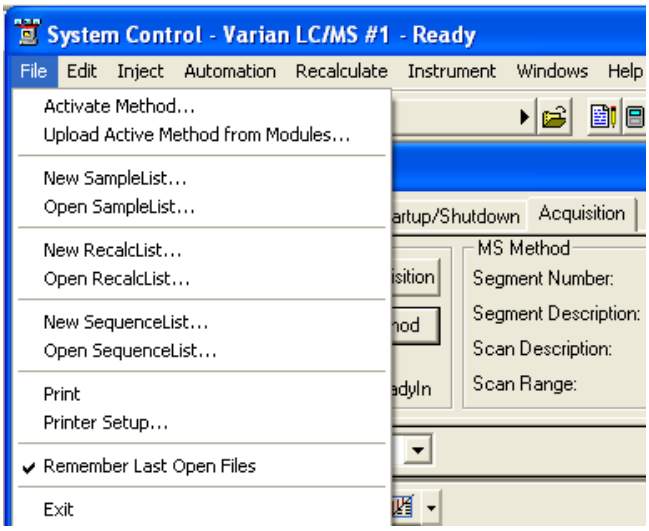
The Varian MS Workstation supports the Varian HPLC modules, the 500-MS CVM, and the LC Combi PAL AutoSampler.

Refer to **Data Acquisition with LC Control** (part number 0391473200) for details on the operation of the Varian MS Workstation with Varian HPLC modules. It covers configuring LC modules in an instrument for data acquisition, building a method, and running samples. This manual is included on the Varian MS Workstation CD-ROM.

System Control Command Reference

System Control Menus

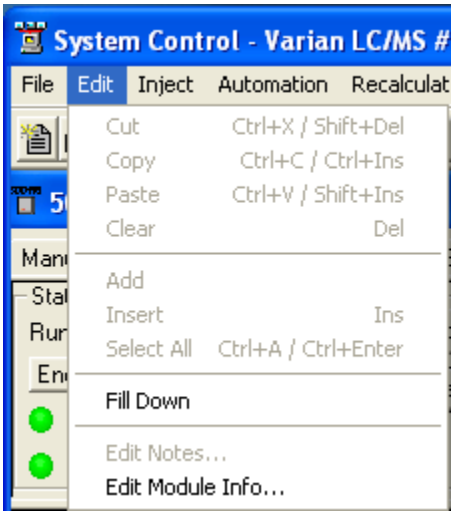
File Menu



Menu Item	Description
Activate Method…	Activate a Method and download the Instrument Control sections to the modules.
Upload Active Method from Modules…	Name the Method that receives the instrument control sections uploaded from all modules connected to the instrument.
New SampleList…	Name a new SampleList.
Open SampleList…	Open an existing SampleList.
New RecalcList…	Name a new RecalcList.
Open RecalcList…	Open an existing RecalcList.
New SequenceList…	Name a new Sequence.

Menu Item	Description
Open SequenceList…	Open an existing Sequence.
Print	Prints the contents of the active window in System Control.
Printer Setup…	Select a printer and set options for it. You can open the Print Setup dialog box from the Star Printer Setup dialog box. The Varian MS Workstation only uses the printer for instrument 1.
Remember Last Open Files	Restore the active Method and any automation files that were open when System Control was last closed upon System Control startup if checked.
Exit	Close System Control. If System Control is in the process of performing a critical operation, you may be prompted before the application is closed.

Edit Menu

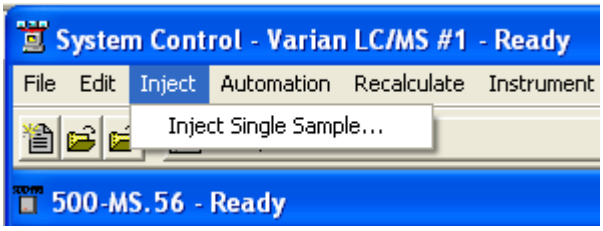


Use these commands with SequenceLists, SampleLists, and RecalcLists.

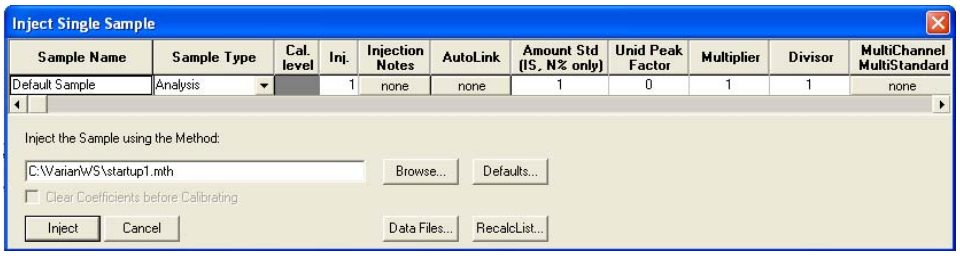
Menu Item	Description
Cut	Deletes a selection and copies it to the Clipboard. Use to remove or move a selected part of a spreadsheet or entered text.
Copy	Copies a selection to the Clipboard. Use to duplicate a selection and place the duplicate in a new place (using Paste).
Paste	Inserts previously cut or copied information stored in the Clipboard into a spreadsheet.
Clear	Deletes a selection but leaves the Clipboard unchanged.
Add	Adds a new line in a SequenceList, SampleList, or RecalcList.
Insert	Inserts a new line in a SequenceList, SampleList, or RecalcList.

Menu Item	Description
Select All	Selects all lines in a SequenceList, SampleList, or RecalcList.
Fill Down	Copies the contents of the top cell in a series of highlighted cells to the cells below it. Use to edit all the cells in a column quickly.
Edit Notes	Opens the Edit Notes Dialog Box to permit editing of the notes associated with the SequenceList, SampleList, RecalcList, or Method file displayed in the active window.
Edit Module Info	Opens the Module Information Editor Dialog Box to permit editing of the Module Information associated with the module displayed in the active window.

Inject Menu

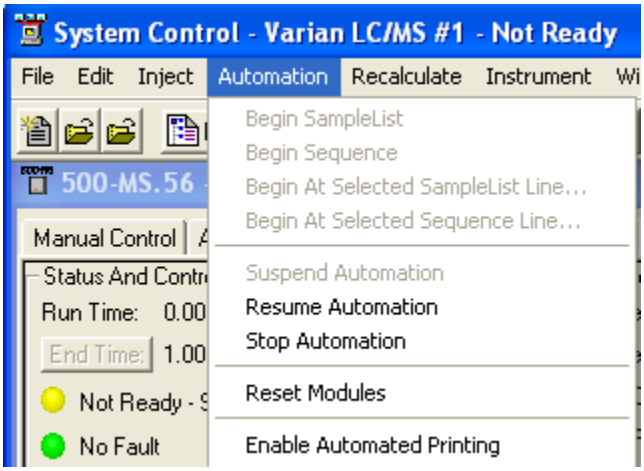


Menu Item	Description
Inject Single Sample	Opens the appropriate Inject Single Sample Dialog Box for your system. Use to inject a single sample without building a SequenceList and SampleList manually.



For more information on the Inject Single Sample dialog, please see “Injecting Samples” on page 96.

Automation Menu



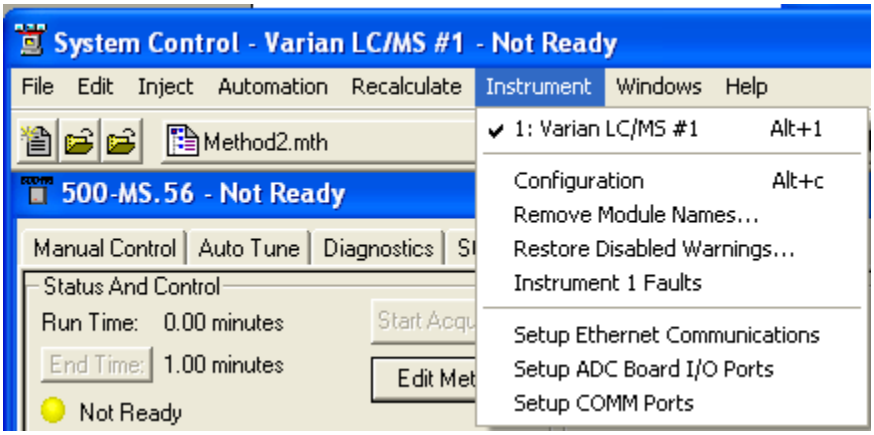
Menu Item	Description
Begin SampleList	Begins executing the currently open SampleList. The currently active method downloads and the system will begin to equilibrate and stabilize.
Begin Sequence	Begins executing the currently open Sequence. The method specified in the first line of the sequence downloads and the corresponding SampleList is activated.
Begin at Selected SampleList Line...	Begins the open SampleList at a selected SampleList line. This item is enabled when a SampleList is open. Upon selecting this menu item, a prompt for the line in the SampleList appears. This is useful when recovering from a power failure that interrupted automation in progress.
Begin at Selected Sequence Line	Begins the open Sequence at the selected Sequence line. This is enabled when a Sequence is open. After selecting this, a prompt for the line in the Sequence appears. It is useful when recovering from a power failure that interrupted automation in progress.
Suspend Automation	Suspends execution of automation after the current run completes.
Resume Automation	Resumes execution of automation after it has been suspended.
Stop Automation	Stops the current run, resets all modules simultaneously, and suspends automation.
Reset Modules	Stops the current run, resets all modules simultaneously. Automation proceeds to the next injection after all the modules go to the Ready state.
Enable Automated Printing	Turns on or off printing of reports automatically generated during any automation action. This is useful when you need to disable printing (if the printer is out of paper, for example) without having to modify the Print Options portion of the Report Method.

Recalculate Menu

System Control - Varian LC/MS #1 - Not Ready
File Edit Inject Automation Recalculate Instrument Windows Help
Method2.mth
500-MS.56 - Not Ready
Begin RecalcList
Begin At Selected RecalcList Line...
View Calibration Curves...

Menu Item	Description
Begin RecalcList	Begins recalculation of the open RecalcList using the active Method.
Begin at Selected RecalcList Line	Begins the open RecalcList at the selected RecalcList line. This item is enabled when a RecalcList is open. After selecting this, a prompt for the line in the RecalcList appears. It is useful when recovering from a power failure that interrupted automation in progress.
View Calibration Curves	Opens the Open a Method File to View Curves dialog box to view calibration curves that are stored in a Method file.

Instrument Menu

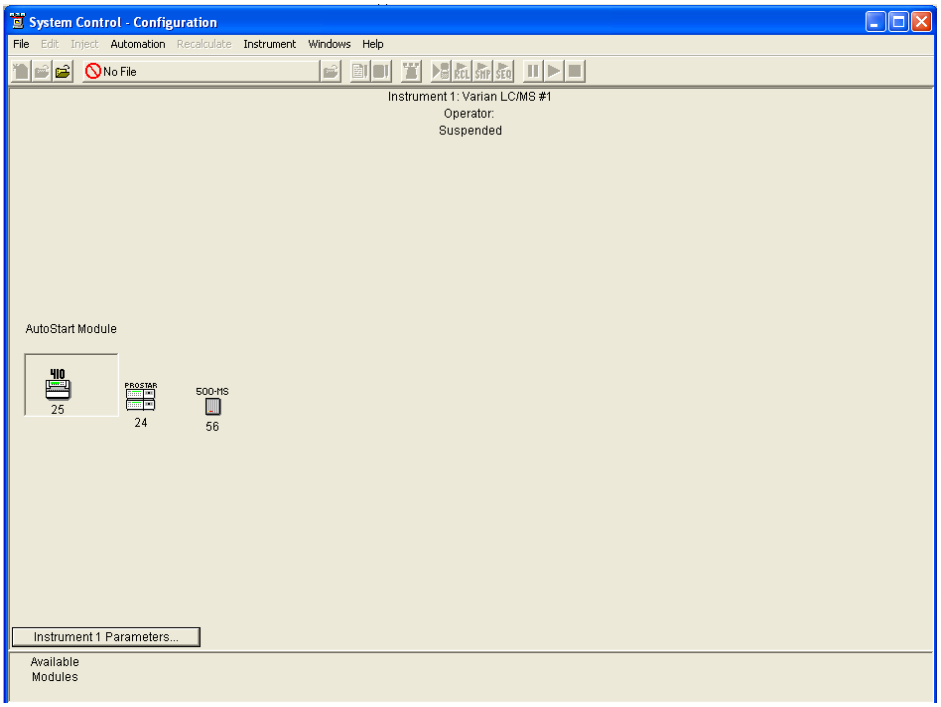


The Instrument Menu commands switch the display between the configuration screen and the instrument.

Menu Item	Description
Instrument Name	Displays the Instrument Status window and Status and Control windows for the named Instrument, which is Varian LC/MS #1 for this example. Enter or edit the Instrument name in the System Control Configuration window.
Configuration	Displays the System Control Configuration window.
Remove Module Names…	Brings up a dialog listing any associations you have set up. You may select items from this list to remove individual associations, in preparation for renaming LC modules.
Instrument # Faults	Displays the Instrument Faults dialog box with recent and self-test messages and faults. The list may be printed from

Menu Item	Description
	this dialog box.
Setup Ethernet Communications	Available if an optional module driver is installed that uses Ethernet to communicate with the device. See the Operation Manual for your module driver for a detailed description, if applicable. Allows set-up of the communication between the 3800 GC and System Control.
Setup ADC Board I/O Ports	Opens the Setup ADC Board I/O Ports Dialog Box to configure the ADC Board base address and determine the Switch S1 settings for each ADC Board. Applicable only if the ADC Board module driver is installed.
Setup COMM Ports	Available if an optional module driver is installed that uses Serial I/O to communicate with the device. See the Operation Manual for your module driver, such as the ProStar/Dynamax pump, for a detailed description, if applicable.

Configuration Window



The Configuration screen is divided into two sections: the Instrument window and the Available Modules window. Initially, modules are displayed in the Available Modules window as they log in, and can be added to the Instrument by clicking and dragging the module icon into the Instrument window. When you open System Control, the system restores the modules in the instrument window that were there when you last closed System Control.

Use the Auto Start Module area to designate which module starts the system in Automation.

- If you are using an AutoSampler, this example uses the ProStar 410 AutoSampler, place it in the AutoStart Module area.

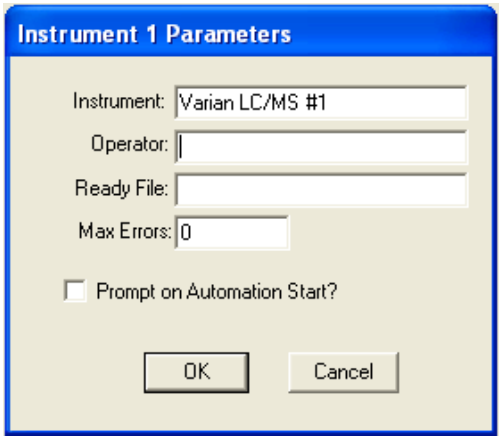
- If you are not using an AutoSampler or other automated injection device, do not place any module in this area.

Enter an **Instrument Name** and **Operator Name** in the Instrument 1 Parameters window.

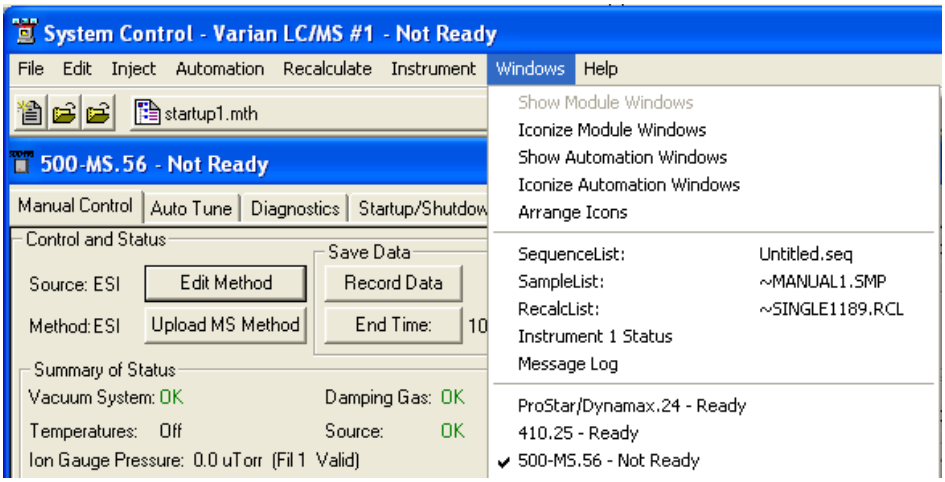
Ready File is used only by earlier versions of a Saturn GC/MS, and should be left blank for operation with 500-MS.

Max Errors is a number that sets the allowable number of non-fatal errors during Automation. When the limit is reached, Automation halts. An entry of 0 disables this feature.

Prompt on Automation Start checkbox allows you to view and edit information in this dialog each time Automation is started.



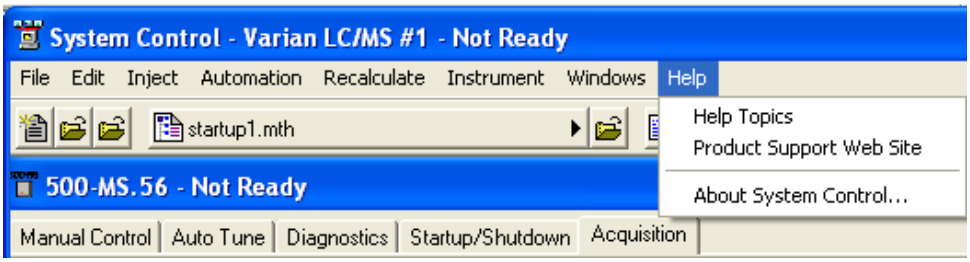
Windows Menu



Menu Item	Description
Show Module Windows	Opens the Status and Control windows for all the modules in the Instrument. It is disabled when all the windows are open, even if some are hidden under others.
Iconize Module	Iconizes the Status and Control windows for all the modules in the Instrument. It is disabled when all the windows are

Menu Item	Description
Windows	iconized.
Show Automation Windows	Displays the windows for all the automation files open in the Instrument. It is disabled when all the automation windows are open, even if some are hidden under others.
Iconize Automation Windows	Iconizes the windows for all the automation files in the Instrument. It is disabled when all the windows are iconized.
Arrange Icons	Arranges the icons, if any, in a row at the bottom of the System Control window.
SequenceList:, SampleList:, RecalcList:	Opens the window for the selected file or moves it to the front. Use to display the open SequenceList, SampleList, or RecalcList.
Instrument Status, Message Log, Configuration Log (only if Configuration window is open)	Displays the corresponding window for the instrument or moves it to the front. Use to see the instrument status, Message Log, or Configuration Log.
Module Window (name varies depending on module)	Displays the Status and Control window for the chosen module or brings it to the front.

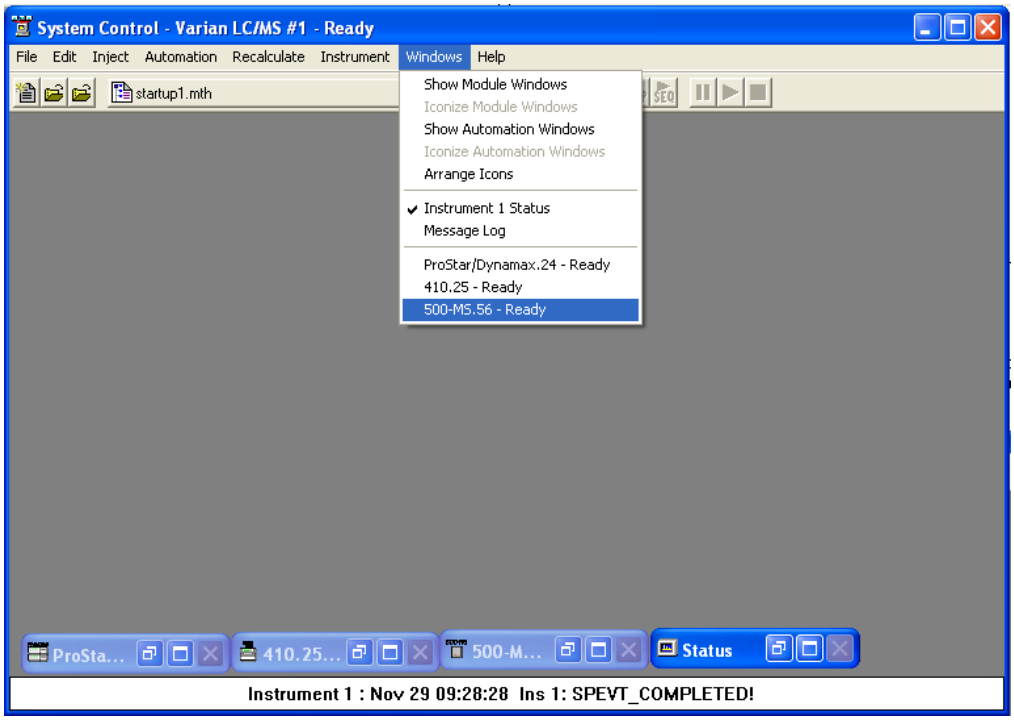
Help Menu



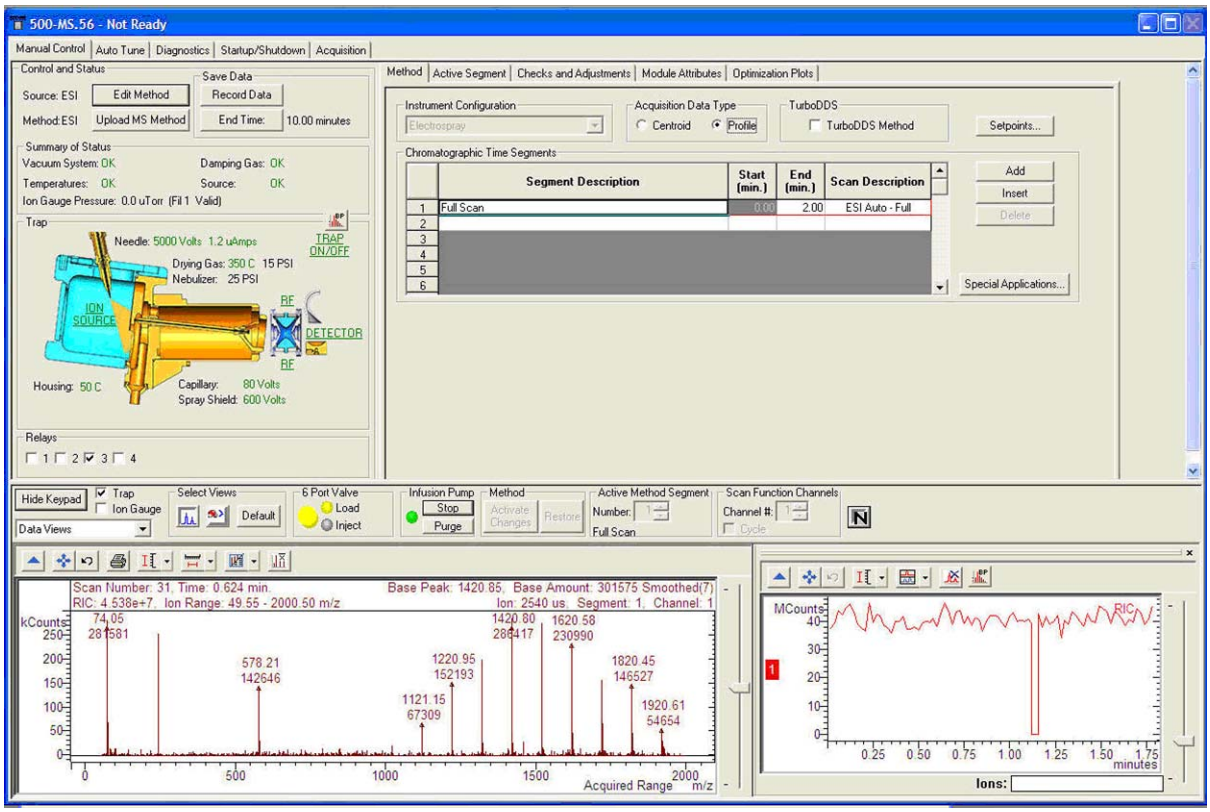
Menu Item	Description
Help Topics	Displays the Help Topics window for System Control.
Product Support Web Site	If you have Internet access and a web browser installed on your computer, this option automatically opens the Varian MS Workstation Product Support Web Site. The latest software and documentation updates for the Varian MS Workstation suite of products, along with additional notes, tips, and answers to frequently asked questions are found here. Visit this site periodically to see if new information is available.
About System Control	Displays the About Box for the System Control application. The About Box contains information about the software version, installation information, and a list of the instrument control modules. You can also validate the installed Workstation files from this dialog.

Display

Display the 500-MS status and control window in the System Control Status window by selecting the Windows menu item and clicking the 500-MS.xx menu item, or by expanding the 500-MS.xx icon that appears at the lower left of the System Control window.



Overview of Tabs



Control and Status Window

Five tabbed sections provide access to 500-MS operations.

Manual Control: adjust all parameters of the currently active method. Global parameters such as Instrument Configuration, Acquisition Data Type, and segment and scan description are set using the Method tab.

Method Segment: adjust some parameters segment by segment.

Checks and Adjustments: adjust the RF ramp.

Module Attributes: set the electron multiplier voltage, trap damping gas flow, syringe type, and the syringe infusion/purge flow rates.

Optimization Plots: optimize the capillary voltage, CID excitation voltage, RF storage level, and needle voltage.

Auto Tune: select and run tuning routines.

Diagnostics tab: run the built in diagnostic routines of the vacuum system and electronics.

Startup/Shutdown: start or shut down the vacuum system and heated zones in an orderly manner.

Acquisition: run samples and acquire data files, with the Inject and Automation menus of the System Control window**.**

Hide/Show Keypad

Click **Hide Keypad** to Hide the Control and Status window. This becomes the Show Keypad button to restore the Control and Status display. While the keypad is hidden, the On/Off controls for the trap and infusion pump are still accessible, as well as the method segment, channel, and intensity normalization selectors.

Manual Control

Manual Control is closely linked with the Method Builder function of the software. A method may be built in Method Builder, activated in System Control, and then manipulated in Manual Control. Conversely, a method may be modified and fine tuned in Manual Control, then uploaded and saved under the same name or as a new named method.

The important distinction is that the effects of changes made in Manual Control are seen immediately in the real time spectrum display, using LC tuning solution ions as a sample.

Control and Status

Control and Status shows a graphic of the ion trap system and status of the needle and capillary voltages, drying and nebulizer gas pressures and temperatures. Click trap in the graphic display to turn on/off the ion source, RF system and the multiplier. Clicking the ion sources, RF, and multiplier turns these parameters on/off individually.

Edit Method

Edit Method opens Method Builder with the method currently active in System Control.

Upload MS Method

Upload MS Method opens a Save Method dialog to allow the MS method parameters currently active in System Control to be copied to an existing method file, or to a new method name. Changes made in Manual Control must be activated using **Activate Changes** before they can be uploaded.

Save Data

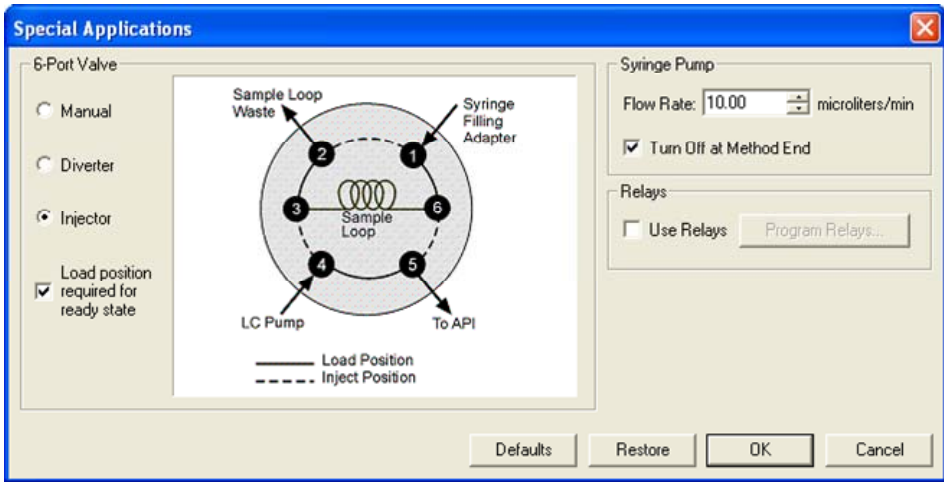
Save Data allows acquisition of data in Manual Control mode, while the ion trap system is turned on by clicking the Trap checkbox. The acquisition time is set by clicking the End Time tab. Once the data acquisition starts by clicking the Start Acquisition tab, it can be stopped by clicking the Stop Acquisition tab. When the acquisition stops, the Create Data File dialog opens for creating a data file name.

Checkboxes

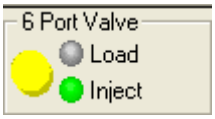
Click the Trap checkbox to turns on the entire ion trap system. Click the **Ion Gauge** to display the pressure inside the trap.

6 Port Valve

This indicates the active solvent/sample flow path for each configuration of the 6 port valve. Select by clicking the **Special Applications** tab in the Method field.



This manual and the Hardware Operation manual have information about the 6 port valve. Click the yellow button to the left of Load and Inject to activate the valve.



The names of the two possible states depend on the selected configuration of the 6 port valve:

The **Manua**l configuration names are Position1 and Position 2.

The **Diverter** configuration names are Divert and MS.

The **Injector** configuration names are Load and Inject.

The 6 port valve state that is active in the Acquisition mode, is controlled on a segment-by-segment basis by checking or un-checking the Divert/Load checkbox under the Active Segment.

Syringe Pump

Use the built-in motor driven syringe to infuse samples into the source while tuning and optimizing electronic and physical settings. You can control two actions: Run and Purge.

Method: Activate Changes/Restore

These buttons become active only after one or more changes have been made through Manual Control. Make a change part of the active method by pressing **Activate Changes**. At any point before this, changes may be reversed and the method segment restored to its previous condition by pressing **Restore**. To make changes permanent, click **Activate Changes**, click **Upload MS Method**, select a folder and file name, and save the method. You are prompted to save any un-activated changes when you leave the Manual Control tab or change Segment number.

Active Method Segment Number

Use the active method segment control to select an existing segment of the active method for review or modification.

Scan Function Channels

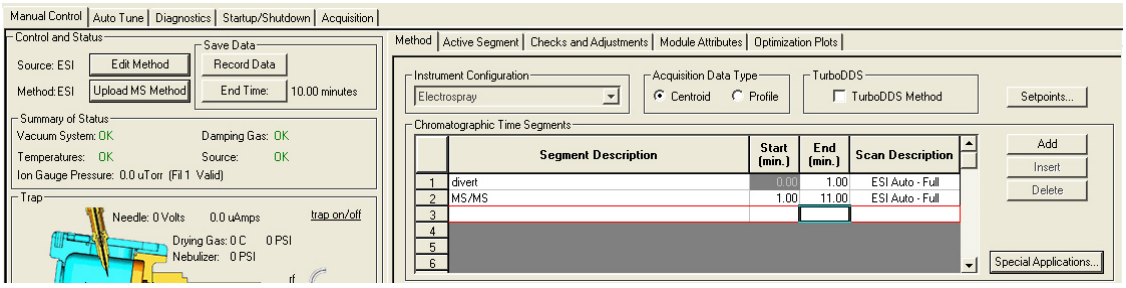
Some of the 500-MS methods have multiple channels. The channels can be thought of as a subset of a segment, where the scan conditions can differ. These are most frequently used for alternating polarity or MRM, work. In Manual Control, you can continuously scan a single channel, or to rotate through them sequentially, as happens in the acquisition mode.

Intensity Normalization

This turns intensity normalization on and off. When it is on, the intensities are normalized by the ionization time. The intensities are multiplied by the ion time ratio. The ion time ratio is the maximum ion time specified, divided by the actual ionization time.

Method

The parameters that you can modify under the Method tab are illustrated in the following for Electrospray Ionization. The Instrument configuration (ESI or APCI) cannot be changed in System Control. These selections must be made through Method Builder.



Acquisition Data Type

Centroid data is the most common acquisition type, as data handling, library searching, quantitation and spectral comparison can be done from this type of data.

Profile data is typically used to determine the charge state of multiply charge ions such as proteins and peptides, quantitation and for diagnostic purposes. Profile files are also approximately 10 times larger, but can be converted to centroid after acquisition.

Chromatographic Time Segments

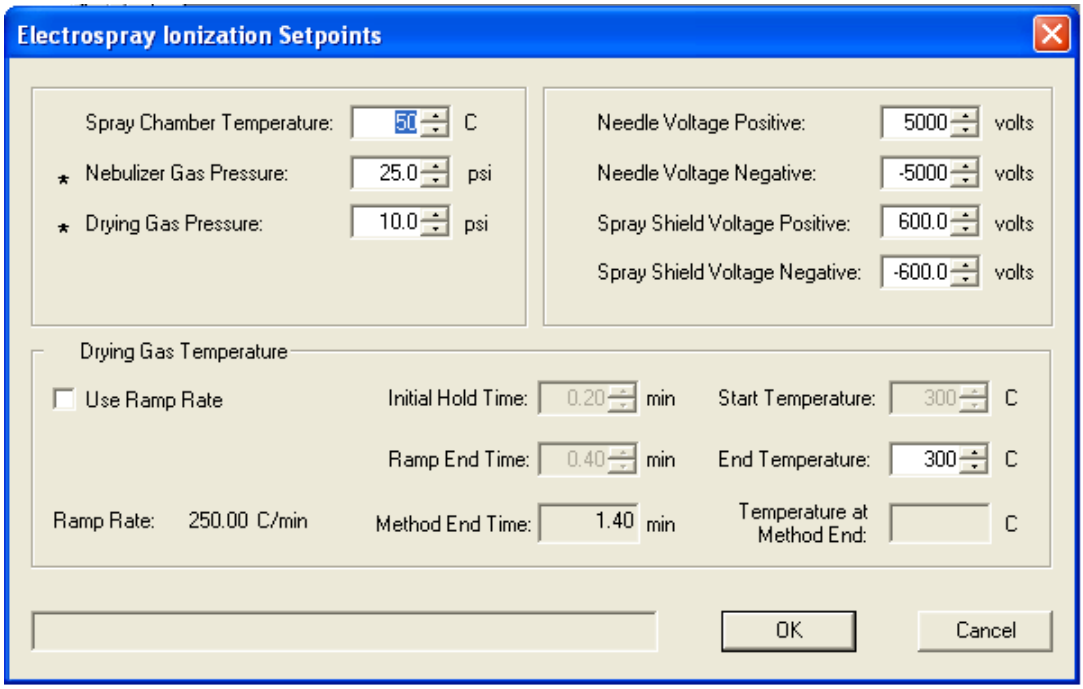
Use the Chromatographic Time Segments to time-program analysis conditions to get the best results for each segment. Time segments up to 1440 minutes in length can be added, inserted, or deleted using the Add, Insert, and Delete tabs. You can acquire a mass spectrum in full-scan with a single analysis segment. Or, you can tailor variables such as acquired mass range, ionization parameters, insert MS/MS segments for individual analytes.

Double-click the required field to edit the **Segment Description**, **Start,** and **End** times. The Scan Description information is automatically entered based on the choice of Scan Type and Ionization mode made in the Method Segment.

Use Special Applications to set the configuration of the 6 Port Valve.

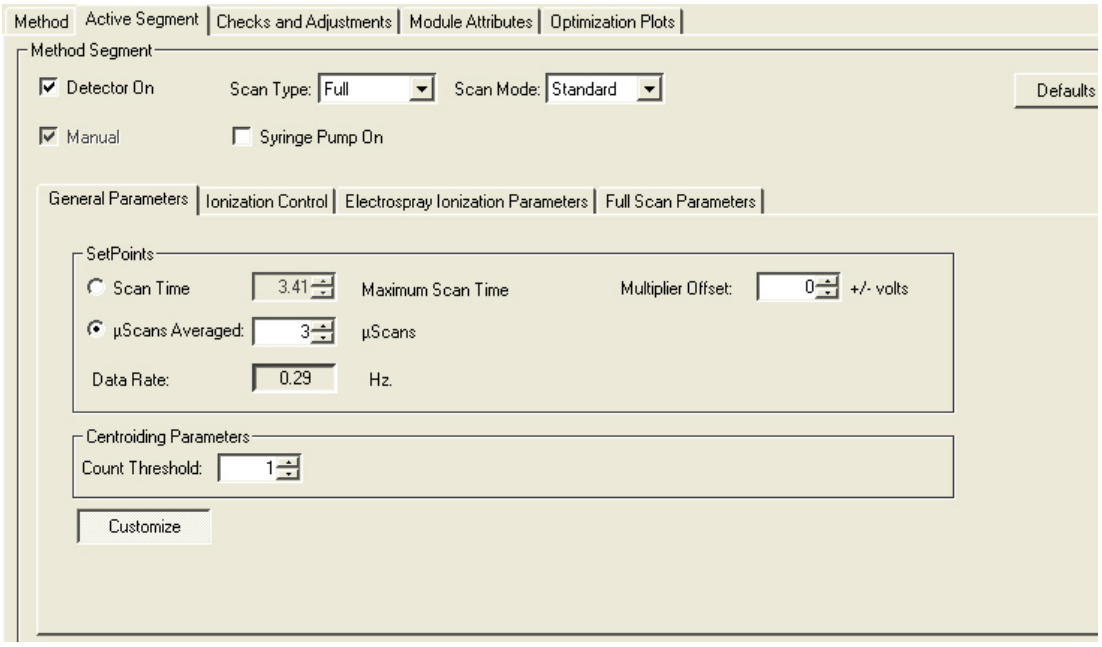
Setpoints

The parameters, which can be modified at the method level by clicking **Setpoints**, are shown for Electrospray Ionization. These settings can be overridden for each segment under the Active Segment section. The actual values of these parameters are displayed in the schematic picture on the left in the 500-MS module window.


Electrospray Ionization Setpoints
Spray Chamber Temperature: 50 C
* Nebulizer Gas Pressure: 25.0 psi
* Drying Gas Pressure: 10.0 psi
Needle Voltage Positive: 5000 volts
Needle Voltage Negative: -5000 volts
Spray Shield Voltage Positive: 600.0 volts
Spray Shield Voltage Negative: -600.0 volts
Drying Gas Temperature
Use Ramp Rate
Initial Hold Time: 0.20 min
Start Temperature: 300 C
Ramp End Time: 0.40 min
End Temperature: 300 C
Ramp Rate: 250.00 C/min
Method End Time: 1.40 min
Temperature at Method End: C
OK
Cancel

Active Segment

The functions in the **Active Segments** tab for ESI.



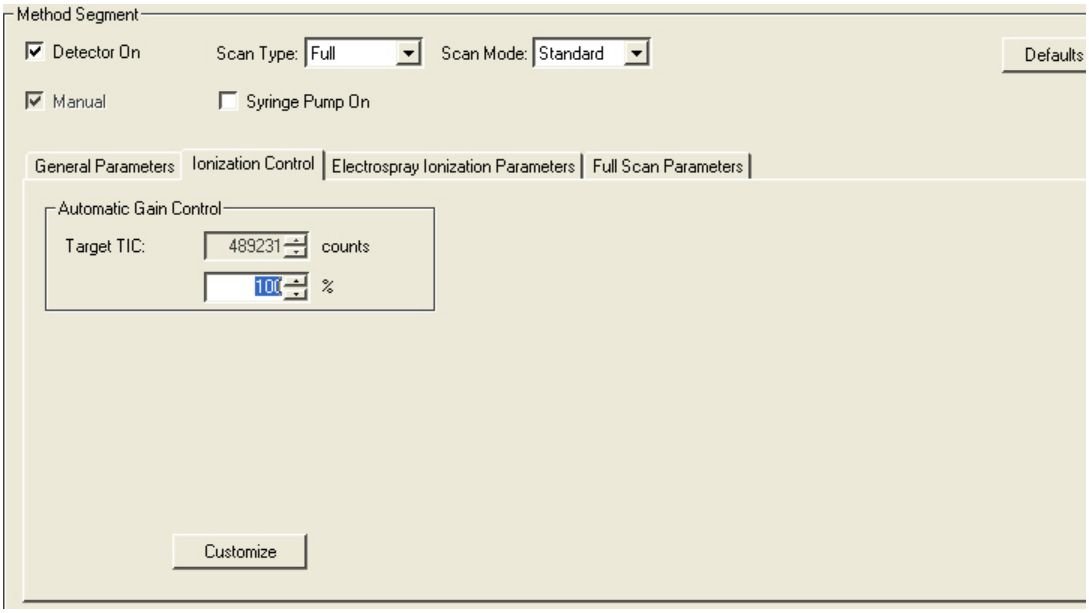
General Parameters: Scan Time and µScans Averaged are linked with each other and with the Scan Type, Ionization, and Scan Mode.

If you adjust µScans Averaged, Scan Time and Data Rate change to values determined by the Scan Type, Ionization mode, and Scan Mode.

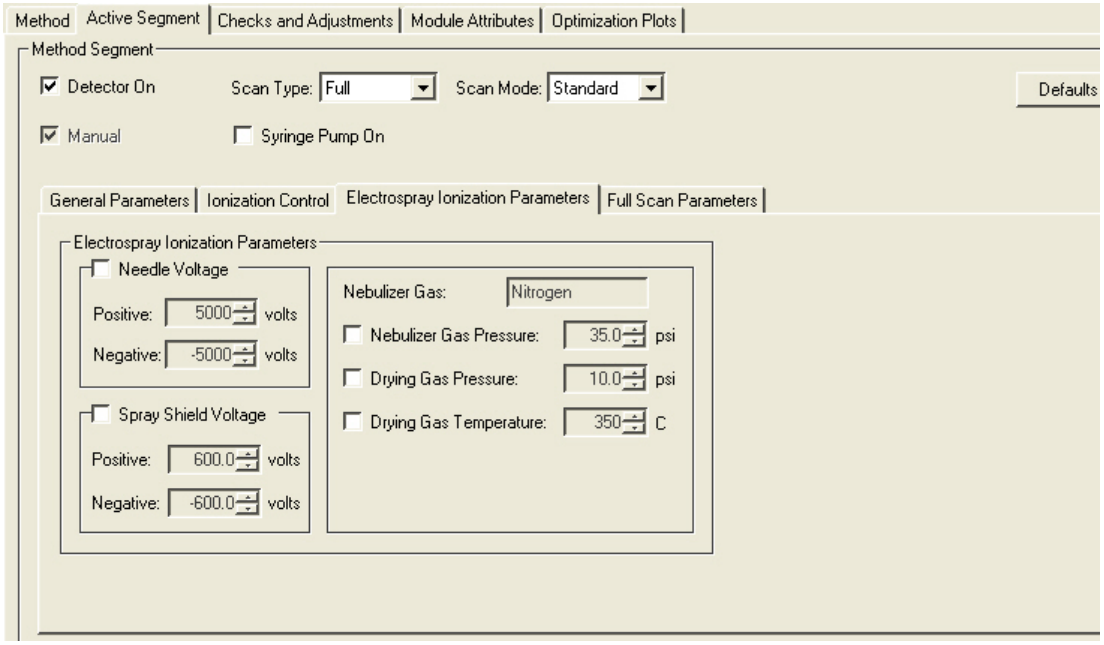
Changing Scan Time changes µScans Averaged and Data Rate.

Select the state of the 6 Port Valve in this section as follows: if the Load box is unchecked, the 6 Port Valve (configured in the Injector mode under the Method tab) is in the Inject state, otherwise it is in the Load state. The name of the 6 Port Valve indicates the function. The syringe pump can be controlled in each segment in this field. Theses setting are active in the Acquisition mode.

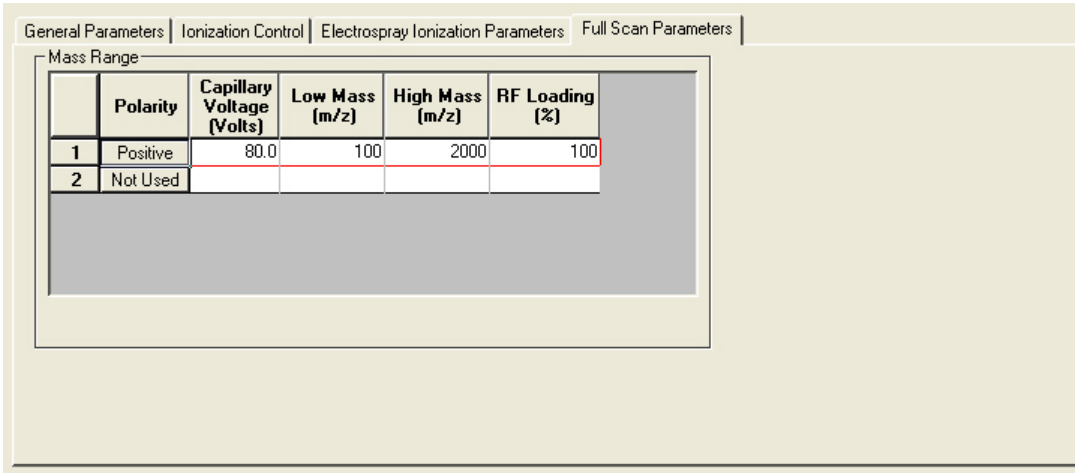
Ionization Control: adjust the Automatic Gain Control (AGC) target that is the total number of ions the system attempts to generate within the trap, up to the Maximum ionization time selected. A lower target reduces space charge effects on resolution, while a higher target may improve quantitation. An optimum value of the target total ion current (TIC) was calculated and should give good results for a broad range of applications. Adjust this by changing the percentage. If the **Fixed Ionization Time** box is checked, the AGC adjustment of ionization time is replaced by the fixed ionization time selected.



Electrospray Ionization Parameters: If checked, the value overrides the setting in the Method Setpoints section.

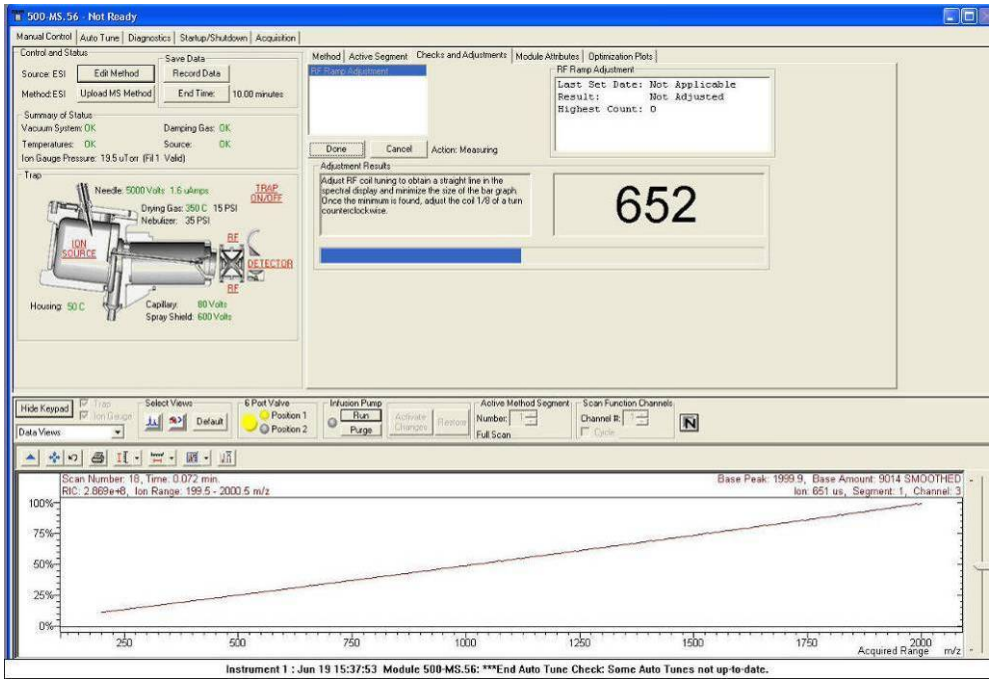


Full Scan Parameters: Select the mass range over which data is acquired. An optimum RF storage level is calculated automatically for the mass range selected. However, you may customize the RF storage level by entering a percentage of this predetermined level. Also, select the polarity of the acquired ions for each segment.



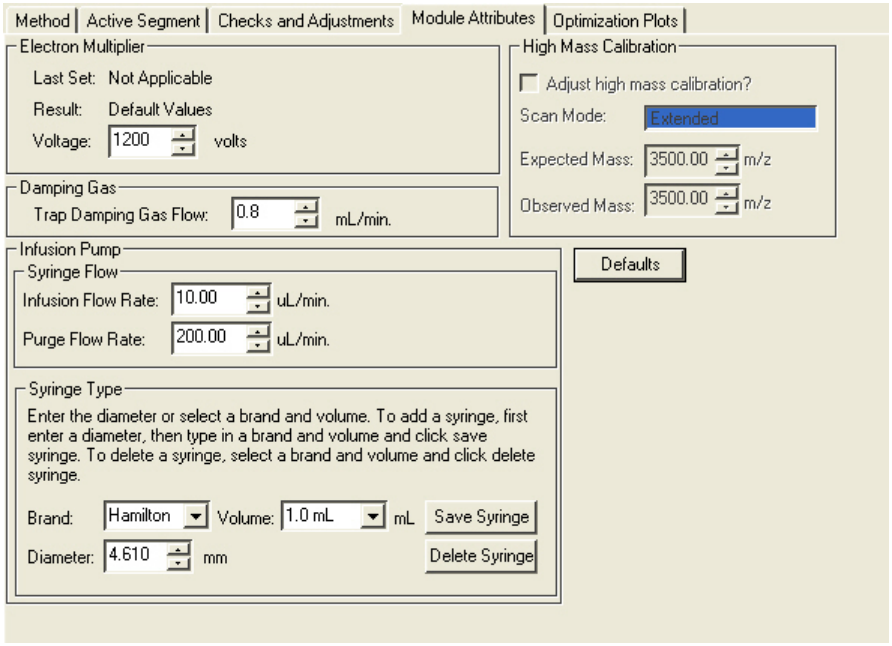
Checks and Adjustments

Adjust the RF ramp. RF voltage ramp is a check of linearity of the RF voltage over the operating mass range of the system. The display ramp should be linear and with minimum amplitude. The RF adjust screw is on the front panel of the 500-MS.



Module Attributes

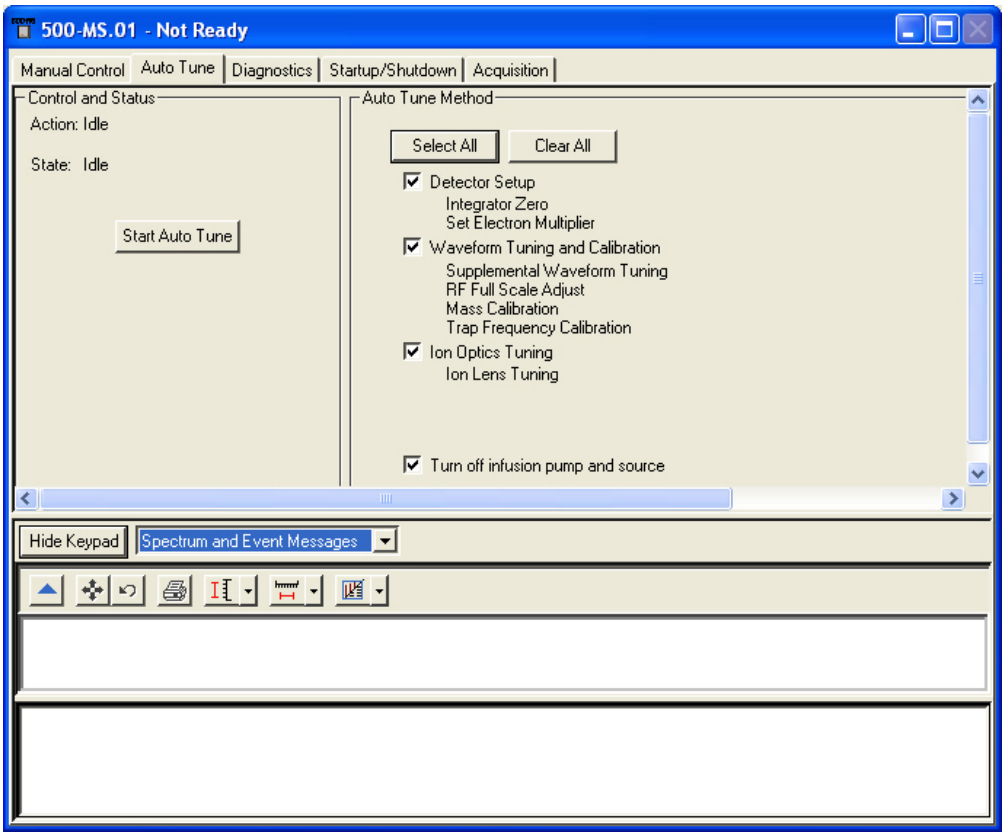
Set the electron multiplier voltage, damping gas flow, the motor-driven syringe attributes and infusion and purge flow rates.


Method
Active Segment
Checks and Adjustments
Module Attributes
Optimization Plots
Electron Multiplier
Last Set: Not Applicable
Result: Default Values
Voltage: 1200 volts
Damping Gas
Trap Damping Gas Flow: 0.8 mL/min.
High Mass Calibration
Adjust high mass calibration?
Scan Mode: Extended
Expected Mass: 3500.00 m/z
Observed Mass: 3500.00 m/z
Infusion Pump
Syringe Flow
Infusion Flow Rate: 10.00 uL/min.
Purge Flow Rate: 200.00 uL/min.
Defaults
Syringe Type
Enter the diameter or select a brand and volume. To add a syringe, first enter a diameter, then type in a brand and volume and click save syringe. To delete a syringe, select a brand and volume and click delete syringe.
Brand: Hamilton
Volume: 1.0 mL mL
Save Syringe
Diameter: 4.610 mm
Delete Syringe

Auto Tune

Auto Tune is an automated process for optimizing the 500-MS performance over the entire mass range. After performing major maintenance, such as cleaning the ion trap, or ion source, run all Auto Tune routines.

If you have the TurboDDS™ option, Auto Tune in TurboDDS mode before acquiring High Resolution or Extended Mass range data files.



The three major sections of the Auto Tune window are Control and Status display, Spectrum and Event Message windows, and Auto Tune Method display.

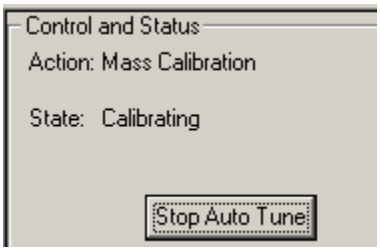
Control and Status

Click **Start Auto Tune** to start the routines.

Click **Stop Auto Tune** to stop them.

The Action field shows the tuning routine in progress.

The State field displays if the 500-MS is currently executing an auto tune routine or is idle.



Spectrum and Event Message Window

Display the AutoTune tests. **Hide Keypad** causes the upper portion of the screen to disappear and the lower portion to expand.

The current Module Attributes has information stored from the last auto tune. When you are finished performing the auto tune, view this message, which is stored in an auto tune log.

```
MS Workstation (Demo) (V6.9.1 03/31/08) Version 6.9.1
Module: 500-MS.1


Configuration
     System Type:                                LC
     Ionization Source:                          Electrospray
```

Auto Tune Methods

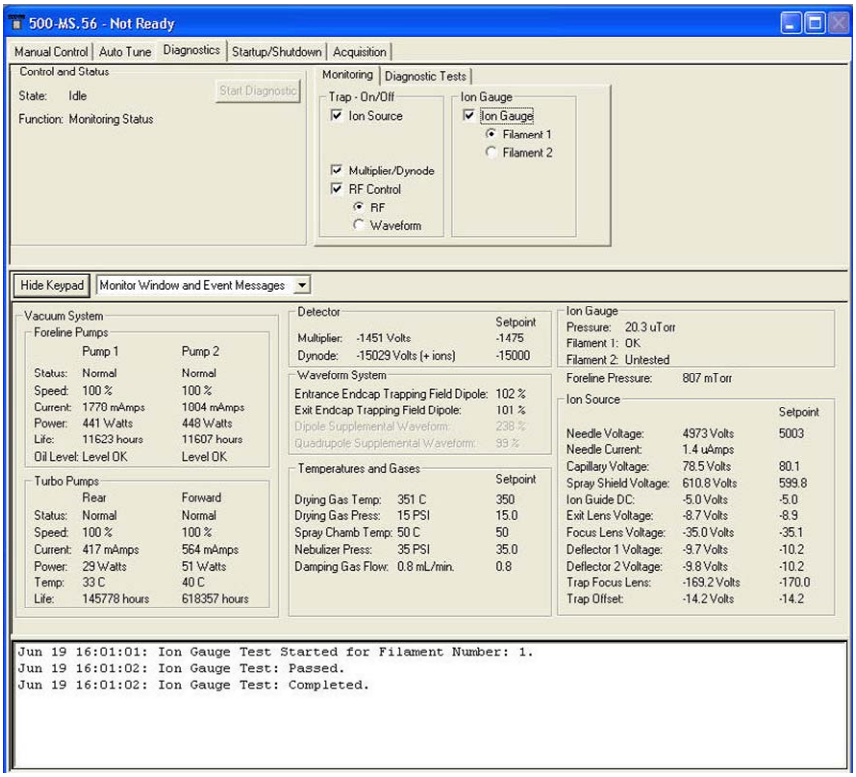
Three auto tune checks automatically set up, tune, and calibrate the mass spectrometer. The last routine is Turn off Infusion Pump and Source. This option is useful for conserving the tuning solution. When this box is checked, the infusion pump and source turn off at the completion of Auto Tune.

Auto Tune routines are performed in the order listed, beginning with Integrator Zero. All of the auto tune methods are selected if you click **Select All** or cleared if you click **Clear All**.

To do Auto Tune, click the desired check boxes and click **Start Auto Tune**. Stop Auto Tune at any time by clicking **Stop Auto Tune**. For a complete description of all the auto tune routines, see the Auto Tune section presented earlier in this manual.

Diagnostics

Control and status for monitoring and diagnosing the Mass Spectrometer.



Control and Status

This indicates the state of the Diagnostics system (Idle or Running) and the function the Diagnostics system is currently doing. The normal function is Monitoring Status but various test functions cycle once the **Start Diagnostics** is clicked.

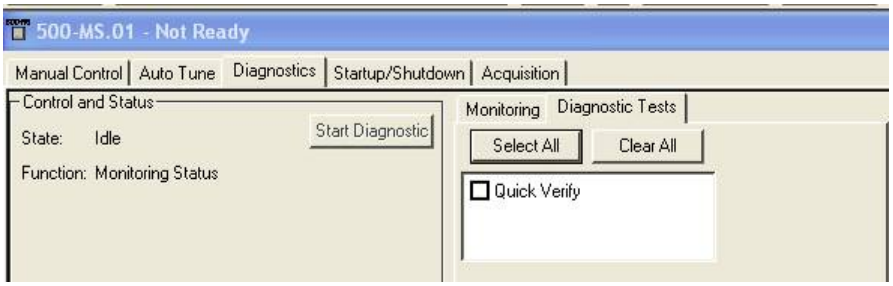
Monitoring Tab

This provides you with choices for monitoring Ion Trap and Ion Gauge components. Selecting the Ion Source checkbox activates the temperature and pressure parameters of the nebulizer and drying gases as well as the needle, capillary and spray shield voltages and ion optics voltages displayed in the Ion Source area (lower Monitor Window section). Selecting the Multiplier/Dynode checkbox activates the Multiplier and Dynode parameters in the Trap data area (lower Monitor Window section). Selecting the RF Control checkbox activates the Waveform System data area (lower Monitor Window section). Underneath the RF Control checkbox, there are two radio buttons (RF and Waveform) that indicate which elements of the Waveform System to monitor. Selecting the RF radio button activates the Entrance End Cap Trapping Field Dipole percentage and Exit End Cap Trapping Field Dipole percentage. Selecting the Waveform radio button activates the Dipole Supplemental Waveform percentage monitor and the Quadrupole

Supplemental Waveform percentage monitor. Selecting the Ion Gauge checkbox adds the Ion Gauge to the components that are monitored and tested. The user may also select which filament is used (Filament 1 or Filament 2) by selecting the appropriate radio button.

Diagnostic Tests Tab

The **Diagnostic Tests** tab has checkbox for **Quick Verify.** This does a system verification check.



Display

Display either the Monitor Window and/or Event Messages by selecting from the menu next to Hide Keypad (Show Keypad). Event Messages are a log of actions and events that have occurred during the Diagnostic and Monitoring process. The messages usually indicate tests Started, Passed, and Completed. The Monitor Window displays the status and values of the following systems: Vacuum, Trap, Waveform, Temperatures and Gases, Ion Gauge, Foreline Pressure, and Ion Source. Display both Monitor Window and Event Messages at the start of any diagnostics and monitoring.

After selecting which components to activate and diagnose, click **Start Diagnostic** in the Control and Status Area. The Monitor Window has the areas that indicate the value and status of components.

Vacuum System

Displays the status of the Turbo and Foreline Pumps. The Turbo Pump area indicates the pump status (Stopped, Running), the pump speed, the pump current (in mAmps) and pump power (in Watts).

Ion Gauge indicates the ion gauge pressure (in µTorr) and the status of the Filaments (OK, open, broken).

Foreline Pressure is indicated in mTorr.

Trap

The Trap area in the Monitor Window is active only if the Multiplier/Dynode checkbox was selected in the Monitoring Tab (top level). The Trap Area (if active) indicates the Multiplier voltage and Dynode voltage (in Volts).

Waveform System

The Waveform System area is active when the user selects RF Control in the Monitoring Tab (top area). The percentages of the Entrance End Cap Trapping Field Dipole and Exit End Cap Trapping Field Dipole are indicated when the RF radio button is chosen underneath the RF Control checkbox.

The percentages of the Dipole Supplemental Waveform and Quadrupole Supplemental Waveform are indicated when the Waveform radio button is chosen underneath the RF Control checkbox.

Temperatures and Gases

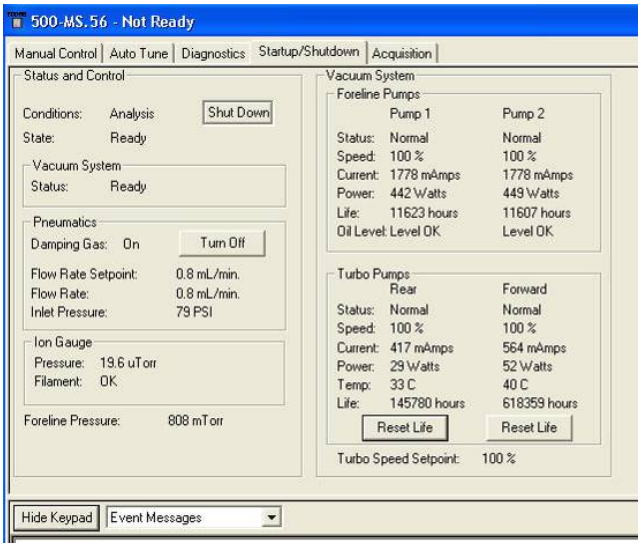
This area indicates the spray chamber temperature, the temperatures, and pressures of the drying and nebulizer gases and the damping gas flow.

Ion Source

The Ion Source Area indicates the readings and setpoint voltages of the needle capillary and all the ion optics components.

Startup/Shutdown

The **Startup/Shutdown** tab provides status and control for the vacuum system, and the damping gas pneumatics.



Status and Control

After the system is shut down, **Startup** opens. Click **Startup** to start the vacuum and turbomolecular pumping.

After the system starts, **ShutDown** opens. Click to begin an orderly shutdown of the vacuum system.

Vacuum System status indicating whether the Vacuum system is Ready.

Pneumatics; Damping Gas Turn On/Turn Off

Use **Turn On/Turn Off** to control the damping gas flow for diagnostic purposes. The flow rate is set in the Manual Control section under the Module Attributes tab.

Vacuum System

Displays the actual conditions of the foreline and turbo pumps.

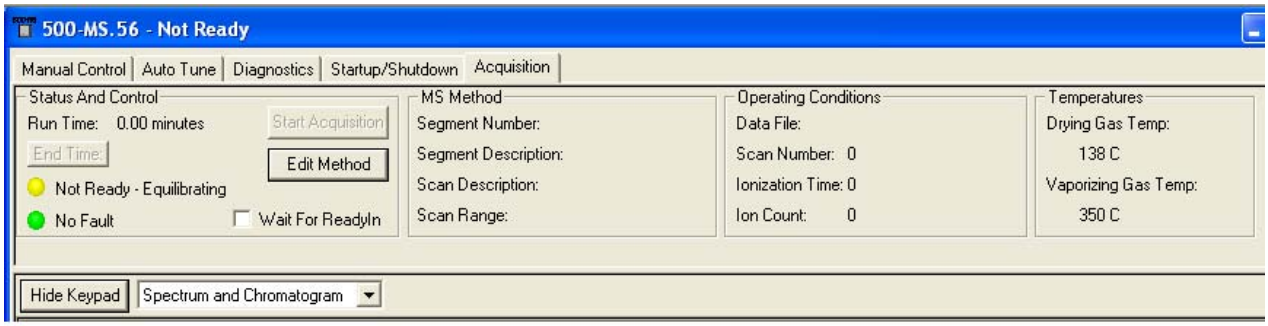
Display

Display either Event Messages or Module Attributes.

- Event Messages are a log of actions and events that have occurred during the Startup or Shutdown process.
- Module Attributes displays the current instrument configuration, temperature setpoints, date and results of the most recent manual adjustments, date and results of the most recent Autotune procedures, and a log of tuning errors.

Acquisition

Selecting the Acquisition tab activates the 500-MS module for analysis and loads the information for the active method.



After a few moments, the red light becomes green indicating that the 500-MS module is ready for analysis. To determine the status ready of each component, go to the top menu under windows to see the states. After all components are ready, you can start an analysis.

Start Acquisition

Click to start data collection with the currently active method.

Edit Method

Open the Method Builder application and load the currently active method.

Wait for ReadyIn

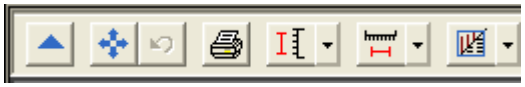
If checked, the state of the **ReadyIn** sync port is included in the checks the system performs to determine if the system is ready. The **ReadyIn** port interfaces to an external device such as a non-Varian Autosampler

End Time

The End Time is initially set to the end time of the active method. You may change the end time to either shorten or prolong the end time of the data acquisition. This does not affect the end time of the LC method.

Status Display

Spectrum Toolbar



Click the green arrow to hide the toolbar. Click in the upper left of the full screen display to restore the toolbar.

Click the full scale button to normalize the spectrum. Double-click the lower left corner of the scale to do the same.

Click the previous scale to restore previous scale.

Click spectrum plot to displays a window previewing the spectrum.

Click scale to select Auto Scale Intensity or Fixed Scale Intensity.

Click mass range to choose Acquired Mass Range, Fixed Mass Range, or Maximum.

Click to choose graphics or a text display of ion intensity and status information.

Chromatogram Toolbar

Hide the toolbar. Click in the upper left of the full screen display to restore the toolbar.

Restores the chromatogram to full scale. Double-click in the lower left corner of the scale does the same.

Restore previous settings.

Select Auto Scale Intensity or Fixed Scale Intensity.

Select display of multiple chromatograms (generated by specific ions entered in the Ions field): Stacked, Overlaid or Normalized.

Clear and restart the chromatogram display.

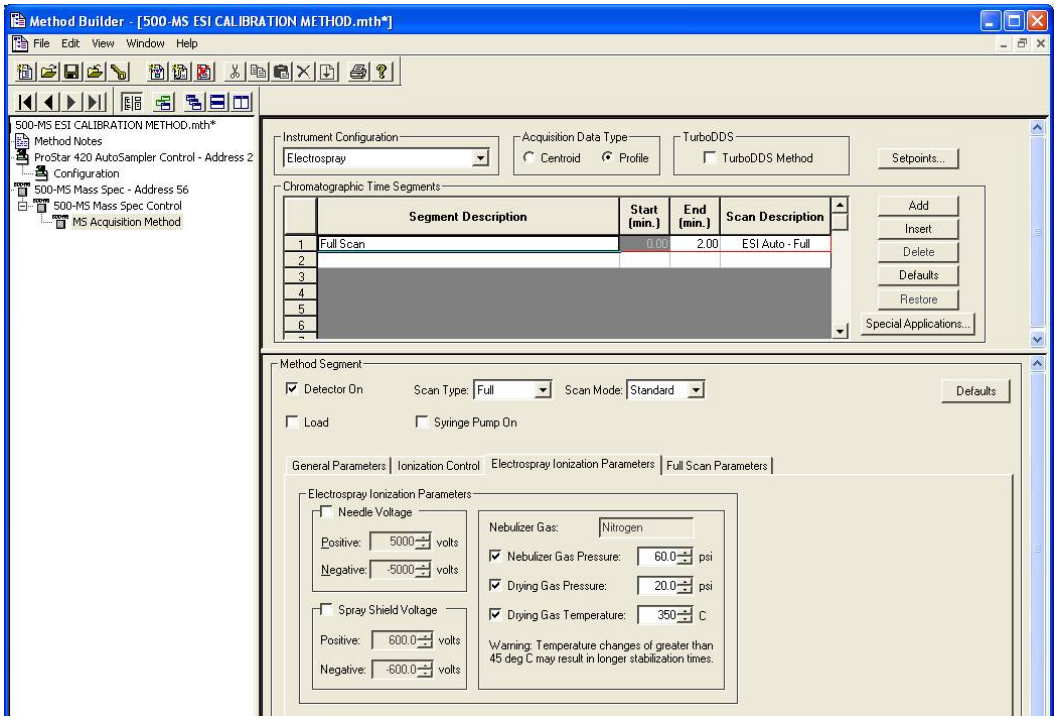
Display a plot of the Intensity of the Base Peak. To display the RIC trace and the Intensity of Base Peak plot at same time, enter IBP in the Ions field. Clicking the Base Peak icon does not affect the display of the extracted ion chromatogram.

Ions

Display chromatograms by entering the m/z values.

Method Builder Command Reference

The available commands depend on the Instrument Configuration and if TurboDDS™ is enabled. In the following graphic, the Instrument Configuration is Electrospray (ESI). The other available Instrument Configuration is Atmospheric Pressure Chemical Ionization (APCI).



Item	Description
Instrument Configuration	System setting. Enable or disable available modules.
TurboDDS Method	If you have this option, you can create TurboDDS methods.
Acquisition Data Type	Either Centroid or Profile. Centroid is the default acquisition data type, as data handling, quantitation, library searching, and spectral comparison can be done from this type of data. Profile data can be used for, TurboDDS data, multiply charged species, quantitation, and for diagnostic purposes. Profile files are also approximately 10 times larger.

Item	Description
Setpoints	Set the ionization source parameters such as temperature and pressure for the nebulizer and drying gases and voltages applied to the components of the ionization source. These parameters are specific for each ionization configuration (ESI or APCI).
Chromato-graphic Time Segments	Time-program analysis conditions for the best segment result. Up to 250 time segments can be created for runs up to 1440 minutes in length. You can tailor variables such as acquired mass range, insert MS/MS segments for individual analytes, and set up the instrument to acquire the best data for each analyte. The time segments program the states of the 6 port valve, which can introduce the sample in to the Atmospheric Pressure Ionization (API) source. Double-click the required field to edit the Segment Description, Start, and End times. The Scan Description information is automatically set based on the choice of Scan Type and Ionization mode in the Method Segment field below.
Segment Description	Describes the nature of the particular segment and usually pertains to a stage in the scan or a name of a compound. Click a Segment Description to update the Method Segment display in the lower window with the details of that segment.
Start (min.)	Indicates the time a particular segment begins. Click to edit the Start Time. The Start Time you enter becomes the End Time of the preceding segment, if one exists.
End (min.)	Indicates the time a particular segment ends. Click to edit the End Time. The End Time you enter becomes the Start Time of the succeeding segment, if one exists.
Scan Description	Indicates the Scan Type and Ionization Type. Edit the Scan Type in the lower Method Segment window to change the scan parameters and update the Scan Description.
Add	Add a chromatographic time segment to the end of the Chromatographic Time Segment Table. Adding a segment copies the parameters from the last segment into the newly created segment. The default duration for the newly created segment is 1.00 minute.
Insert	Inserts a chromatographic time segment before the currently selected row of the Chromatographic Time Segment table. Inserting a segment copies the parameters from the currently selected segment into the newly created segment. Inserting a segment essentially splits the currently selected segment into two segments of equal duration.
Delete	Deletes one or more selected segments from the Chromatographic Time Segment table. However, there must always be one segment in the method. To delete multiple segments, select the entire row for each segment.
Defaults	Sets the selected segment to default values for the selected instrument configuration.
Restore	Restores a chromatographic time segment values to those from the original method file or from the last time the file was saved within the current editing session. Restore is only valid for saved or existing method files. For a new method file, use the Defaults button to set the segment values to default values.
Special Applications	Select the mode for the 6 port valve, and control the syringe pump and relays.

Item	Description
Method Segment	Control which MS scan function is performed: Full scan, AMD, MS/MS, or MS^n. For each Scan Segment selected in the upper Chromatographic Time Segments table, you see menus and tabs that provide the options to create the method. View all the available options if the Detector On button is selected.
Detector On	If the Detector On is not enabled, the ions are deflected off the trap by the ion optics components and the RF voltage and multiplier are OFF during the segment. NOTE: For all data acquisition segments, the Detector On box must be enabled.
Scan Type	There are four Scan Types: Full, AMD, MS/MS, and MS^n. Select a scan type to load its default values into the Parameters and Control tabs in the lower section.
Scan Mode	The four options are Standard, Enhanced, HiRes, and Extended.
Manual / Divert / Load	Control the 6 port valve state for each mode of operation, selected by using the Special Applications tab, and is named accordingly. When the 6 port valve is configured in the Manual mode, this checkbox becomes inactive and the active flow path is controlled in the Manual Control section of System Control. These settings are active in the Acquisition mode.
Syringe Pump On	Use the Syringe Pump On check box, in Acquisition mode, to turn the syringe pump on or off. Change the flow rate in Manual Control.
General Parameters (Tab)	Edit the Set Points for a particular time segment. There are five set points: Scan Time, Scans Averaged, Data Rate, and Multiplier Offset. Count Threshold is available when the Customize button is selected.
Scan Time, µScans Averaged, Data Rate	Scan Time, µScans Averaged and Data Rate are linked. The number of scans averaged is updated when the scan time is adjusted and the reverse is true. Set the scan time by first setting the mass range from the Scan Parameters tab and then changing the scans averaged to three. Three scans averaged gives the best compromise between a high chromatographic data rate and good spectral averaging. San Time Range: 0.47 - 30 seconds (full range) µScans Averaged Range: 1 to 99
Multiplier Offset	Adjusts the EM voltage as much as ±300V relative to the current multiplier setting in the Module Attributes tab dialog in Manual Control (usually the 10^5 gain value from Auto Tune). Sometimes better sensitivity is achieved, particularly in techniques such as MS/MS, when the multiplier voltage is increased. This adjustment can be made for each segment. Use the same multiplier gain for determining the calibration curve and analyzing unknown samples. Range: -300 to +300 volts
Ionization Control (Tab)	Has either 1 or 2 editable fields depending on the Scan Type selected. The Automatic Gain Control Target TIC (Total Ionization Count) always appears, with differing default values depending on the Scan Type. When either AMD or MS/MS is selected, you can set the Prescan Ion Time (in microseconds) for that particular time segment. Also, you may click Customize, which displays additional options to configure Fixed Ionization time, Max Ionization Time and the Prescan Ion Time, if applicable.

Item	Description
Automatic Gain Control Target TIC	Uses the ion count from a prescan at a fixed ion time, along with this target value, to calculate an ion time necessary to fill the ion trap with the target number of ions during the analytical scan. The objective is to fill the trap with an optimal number of ions during each analytical scan. Usually, the Target TIC is not set below 10000 for full scan acquisitions, but it should also not be set too high or spectral distortions due to space charge (loss of MS resolution and/or shift in mass assignments for strong chromatographic peaks) may result. An optimum value of the AGC Target TIC (Total Ionization Count) is predetermined for each Scan Type. You may customize this parameter by changing the percent of its predetermined value. This component is disabled if you choose the Fixed Ion Time checkbox in the Customize area.
Fixed Ionization Time	Generally used for diagnostic tests and not advised for qualitative or quantitative analysis in the ion trap. Choosing this disables both the Automatic Gain Control AND the Prescan Ion Time functions and uses the Fixed Ionization time you entered.
Max Ionization Time	Changing the Max Ionization time can improve the sensitivity of techniques such as MS/MS. If the ion time in the baseline of the chromatogram is at the maximum ion time, the ion trap may not be filled with ions to the level designated by the Target TIC. The number of ions in the trap can be increased by increasing the Maximum Ionization Time. If you take the second approach and are collecting data for quantitation, make sure that enough data points are collected across the chromatographic peaks for reliable quantitation. At least five, but preferably seven or more data points are needed for good quantitative data. The ion signal (but not the number of ions in the trap) can be increased by raising the multiplier voltage. Range: 10 - 650000 microseconds
Electrospray Ionization Parameters	Override the ionization method parameters edited under the Setpoints tab, for each segment.
<Scan Type> Parameters	Appearance depends on the Scan Type selected. For example, if AMD is the Scan Type selected, then the tab reads AMD Parameters. If Full is the selected Scan Type, then Full Scan Parameters.
Full Scan Parameters	Change the values of the Full Scan Parameters Low Mass (m/z) and High Mass (m/z). Also, change the capillary voltage and the polarity for each segment. These two parameters appear for all scan types. When polarity is selected in each row, the polarity switches from one scan to the next resulting in data files with two channels, each corresponding to the selected polarity. An optimum value of the RF storage level is predetermined, however it can be customized by changing the percent value in the RF Loading (%) field.
AMD, MS/MS, MS^n Scan Parameters	Select one of these MS/MS type ion preparation modes, to see a table that has all available MS/MS parameters. Each MS/MS (isolation, dissociation, and scanning) step follows across the table.
Precursor Ion	Enter exact fractional masses. Range: 50 - 2000 m/z

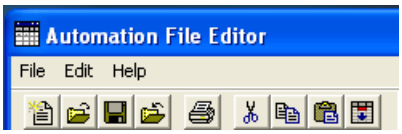
Item	Description
Isolation Window	The full mass isolation window range is 1.0 to 14.0 m/z. The actual range depends on the precursor ion. The default value is 3.0 m/z. Both integral and fractional mass isolation windows are accepted. When first developing a method, start with a mass isolation window of 3 m/z to ensure that the ion is centered in the window. For example, given a precursor ion mass of 1522 m/z and a mass isolation window of 3 m/z, ions of masses 1521, 1522, and 1523 m/z are isolated. If there is a need to reduce the window because of interfering ions, do it in a later step because the customized parameters may require optimization. Some ions are easily dissociated during the isolation step. Increasing the width of the isolation window reduces the ion loss by premature dissociation at the expense of selectivity. Range: 1.0 - 14.0 m/z (full range)
Waveform Type	Select non-resonant or resonant excitation. The non-resonant method is usually the first choice because of its simplicity. Resonant excitation involves exciting the target ions by an RF field of the resonant frequency of the trapped ions. Non-resonant excitation involves exciting the precursor ions using an RF field not at the resonant frequency of the trapped ions.
Excitation Storage Level (m/z)	The RF storage level in m/z while the dissociation waveform is applied following isolation. The Excitation Storage level depends on the precursor mass, but the lowest product ion must be more than 2 mass units above the excitation storage level. The excitation storage level for the precursor ion can be calculated using the “q” calculator. The “q” calculator is accessed by right clicking on any of the fields in the MS/MS parameters table. NOTE: Set the excitation storage level to avoid ejection of the lowest mass product ion. If, for example, a precursor ion of 403 m/z dissociated to product ions with m/z values of 350, 200, and 131, do not use an excitation storage level above 126 m/z.
Excitation Amplitude (volts)	Non-resonant excitation amplitude range: 0-119.58 volts, default 20 Resonant range: 0-59.79 volts, default 0.2 volts
Product Ion Start Mass (m/z), Product Ion End Mass (m/z)	Set the Product Ion Start and End Masses (or Low and High masses) to cover the mass range required.
More Ranges	You can have multiple Product Ion Start and End Masses in a single segment. Click More Ranges to display the Product Ion Mass Ranges Dialog box with a Mass Ranges table, and buttons to Add, Insert, and Delete Product Ion Start and End Masses.
Add	Adds a line to the end of the Mass Ranges Table.
Insert	Inserts a line after the currently selected row of the Mass Ranges table.
Delete	Deletes one or more selected rows from the Mass Ranges table.

Item	Description
“q” Calculator	In scientific literature, storage RF values are often reported in terms of the Mathieu “q” parameter. The value of the “q” parameter determines the stability of the precursor ion trajectory. More stable trajectories allow higher excitation voltages to be applied before ions are ejected from the trap. It has been empirically observed that a “q” value of 0.4 provides an optimum yield of product ions. This calculator determines the corresponding CID storage RF value (m/z) following user entry of an ion mass (m/z) and desired “q” value.
Customize	Displays parameters to customize user settings for set points, ionization parameters, or scan function parameters.
Isolation Waveform	Applied while the trap fills and ejects unwanted ions.
RF Loading (%)	This is the m/z value at which the RF is maintained during ionization and the coarse isolation step. An optimum value is predetermined for each precursor ion. Customize this value by setting a percent of the predetermined value. Range: 0 - 300%
Low Offset (Low Edge Offset)	The Low Edge Offset is the mass offset to optimize the ejection of the mass just below the precursor ion mass. The default value is 0. Increasing the mass offset increases the isolation window on the low mass side of the precursor ion. Decreasing the offset decreases the window on the low mass side. Adjust the offset to minimize the amplitude of the adjacent masses below the precursor ion. Initially, adjust in 0.2 m/z increments. Range: -0.7 to 0.5 m/z
High Offset (High Edge Offset)	Click Customize to view. The High Edge Offset is the mass offset to optimize the ejection of the mass just above the precursor ion mass. The default value is 0. Increasing the mass offset increases the isolation window on the high mass side of the precursor ion. Decreasing the offset decreases the window on the high mass side. Adjust the offset to minimize the amplitude of the adjacent masses below the precursor ion. Initially, adjust in 0.2 m/z increments. Range: -1.0 to 1.0 m/z
High Mass Ejection Factor (%)	Click Customize to view. The High Mass Ejection is the amplitude of the broadband waveform used to eject masses above the isolated precursor ion. An optimum value has been predetermined, but you can enter a percent of it. Range: 0 - 200 %
Excitation Time (msec)	Click Customize to view. The Excitation Time is the time allotted for collision-induced dissociation (CID) by ion excitation. The default excitation time is 10 msec for both resonant and non-resonant excitation. Range: 0 - 650 msec
Modulate RF	Click Customize to view. This parameter (checked = true) specifies whether the RF storage field is modulated during resonant CID. This modulation greatly reduces the effect of changing secular frequency with concentration.

Item	Description
Number of Frequencies	Click Customize to view. This is the number of frequencies at which the CID excitation waveform is applied. The default value is 1 frequency applied at the end caps. A value of 3 generates three frequencies: the resonance frequency and frequencies 500 Hz above and below that frequency. Range: 1 - 119 frequencies (odd numbers only)
Defaults	Sets the parameters of a method segment to their default values. Generally, any user-modified mass values are preserved.
Count Threshold	Appears in the General Parameters Customize option of all Scan Types. Any ions with intensity less than the selected Count Threshold are regarded as noise and are not saved during the run of centroid spectra.

Automation File Editor Command Reference

Menus



File Menu

Menu Item	Description
New	Displays RecalcList, SampleList, and Sequence. Select one to display Create a New Automation File.
Open	Displays RecalcList, SampleList, and Sequence. Select one to display the Open an Automation File dialog box to open an existing automation file of the selected type.
Save	Saves the currently displayed automation file with the existing name.
Save As	Displays the Save Automation File As dialog box. You can save the currently displayed automation file with a new name.
Print	Prints the currently displayed automation file.
Printer Setup	Opens the Print Setup Dialog. You can also open the Print Setup dialog box from the Printer Setup dialog box.
Exit	Quits the Automation File Editor. If you have not saved changes to open files, you are prompted to do so.

Edit Menu

Menu Item	Description
Cut	Deletes a selection and copies it to the Clipboard.
Copy	Copies a selection to the Clipboard.
Paste	Inserts previously cut or copied information that was stored in the Clipboard into a document.
Clear	Deletes a selection but leaves the Clipboard unchanged.
Add	Adds a new line in a SequenceList, SampleList, or RecalcList.
Insert	Inserts a new line in a SequenceList, SampleList, or RecalcList.

Menu Item	Description
Select All	Selects all lines in a SequenceList, SampleList, or RecalcList.
Fill Down	Copies the contents of the top cell in a series of added or existing, highlighted cells to the cells below it. Use to edit all the cells in a column quickly.
Edit Notes	Opens the Edit Notes dialog box to permit editing of the notes associated with the currently open automation file.

Help Menu

Menu Item	Description
Help Topics	Displays help selected topic.
Product Support Web Site	If you have Internet access and a web browser installed on your computer, this automatically opens the Varian MS Workstation Product Support Web Site. This has the latest software and documentation updates for the Varian MS Workstation suite of products, along with additional notes, tips, and answers to frequently asked questions.
About Automation File Editor	Displays the About Box for the Automation File Editor application. The About Box contains information about the software version, installation information, and a list of the instrument control modules that you have installed. You can validate the installed workstation files from this dialog.

Main Toolbar

New Automation File: Select RecalcList, SampleList, or SequenceList. Select one to display the Create a New Automation File dialog box for naming a new file of the selected type.

Open Automation File: Select RecalcList, SampleList, or SequenceList. Selecting one displays the Open an Automation File dialog box to open an existing automation file of the selected type.

Save Automation File: Saves the currently displayed automation file with the existing name.

Save Automation File As: Displays the Save Automation File As dialog box, allowing you to save the currently displayed automation file with a new name.

Print Automation File: Prints the currently displayed automation file.

Cut: Deletes a selection and copies it to the Clipboard.

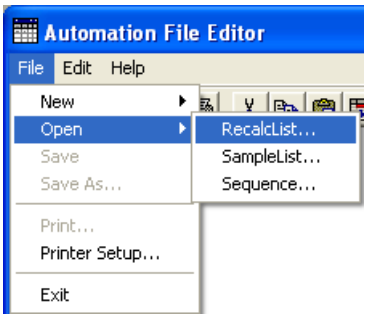
Copy: Copies a selection to the Clipboard.

Paste: Inserts previously cut or copied information that was stored in the Clipboard into a spreadsheet.

Fill Down: Causes the contents of the top cell in a series of highlighted cells to be copied to the cells below it. Use to edit all the cells in a column quickly.

Open Automation File Dialog Box

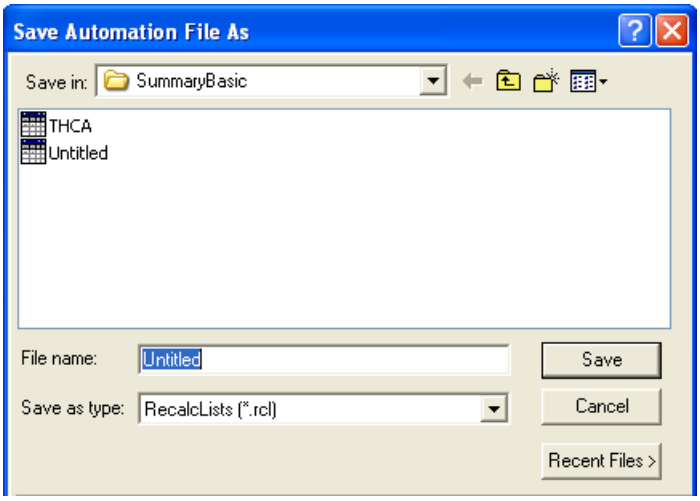
Specify or open an automation file. The Open Automation File dialog box appears with several titles, depending upon your selection: RecalcList, SampleList, or Sequence. It has the following fields in forms appropriate for the type of file you are about to open.



Item	Description
Look in	Lists the available folders and files. To see how the current folder fits in the hierarchy on your computer, click the down arrow. Click a folder to see its contents. The box below shows the folders and files in the selected location. You can also double-click a folder or file in that box to open it. To open the folder one level higher, click the up arrow button on the toolbar.
File list	Lists the folders and files in the selected location. Double-click a folder to see its contents. You can also use the Look In box to see the hierarchy of folders. To open the folder one level higher, click the up arrow button on the toolbar.
File name	Shows the currently selected file.
Files of type	Restricts the list of files to only those matching the selected type.
Recent Files	Click this to display a list of recently selected files. When you select a file from this list, its name opens in the File name box.
Open	Opens the selected file.
Cancel	Cancels file selection.

Save Automation File as Dialog Box

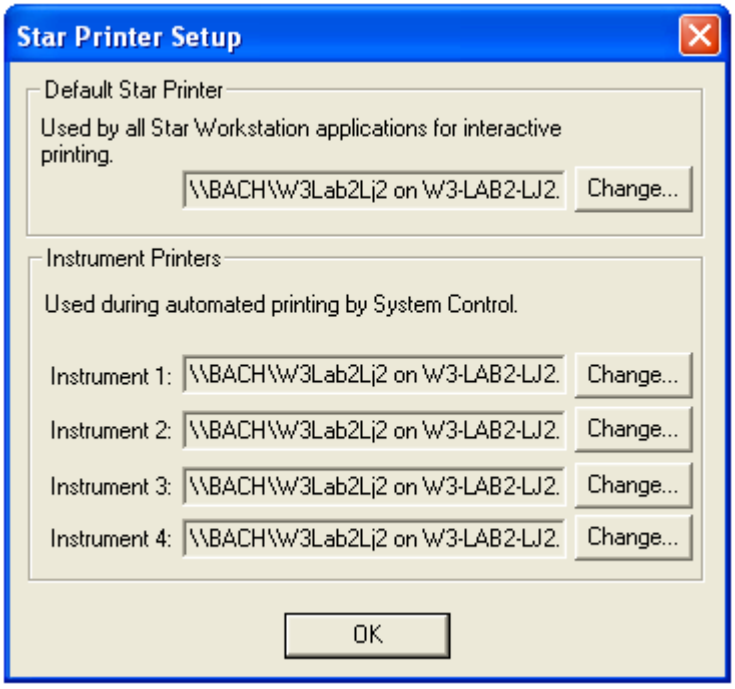
Name an automation file. Access the Save Automation File As dialog box with the menu command File> Save As.



Item	Description
Save in	Lists the available folders and files. To see how the current folder fits in the hierarchy on your computer, click the down arrow. Click a folder to see its contents. The box below shows the folders and files in the selected location. You can also double-click a folder or file in the box to open it. To open the folder one level higher, click the up arrow button on the toolbar. To open a new folder in the current directory, click the Create New Folder button.
File list	Lists the folders and files in the selected location. Click a folder to see its contents. You can also use the Save In box to see the hierarchy of folders. To open the folder one level higher, click the up arrow button on the toolbar.
File name	Shows the currently specified file.
Save as type	Specifies the type of file to save.
Recent Files	Click to display a list of recently selected files. When you select a file from this list, its name is displayed in the File name box.
Save	Saves the file with the specified name.
Cancel	Cancels file saving.

Print Setup Dialog Box

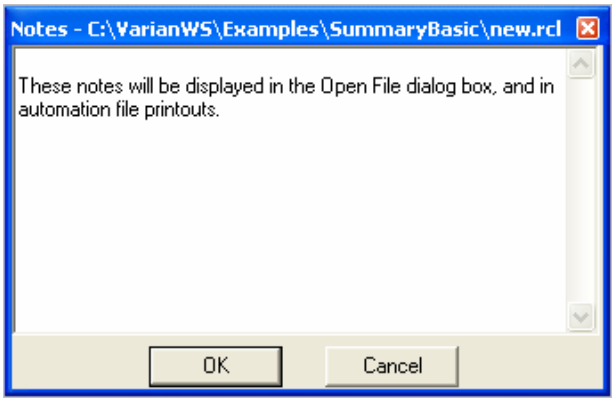
Specify the printers used by Varian MS Workstation applications both interactively and during automation. Select File > Printer Setup…



Item	Description
Default Printer	Displays the printer used when printing interactively from Varian MS Workstation applications. Click Change to select a different printer.
Instrument Printers	Displays the printer used when printing under automation from System Control. Since the Varian MS Workstation is a single instrument workstation, the printer specifications for instruments 2-4 are ignored.

Edit Notes Dialog Box

Access this with the menu command Edit>Edit Notes, while a RecalcList is opened. It allows you to enter descriptive notes associated with an automation file. These notes can be viewed when selecting the file, and are included in the printout.

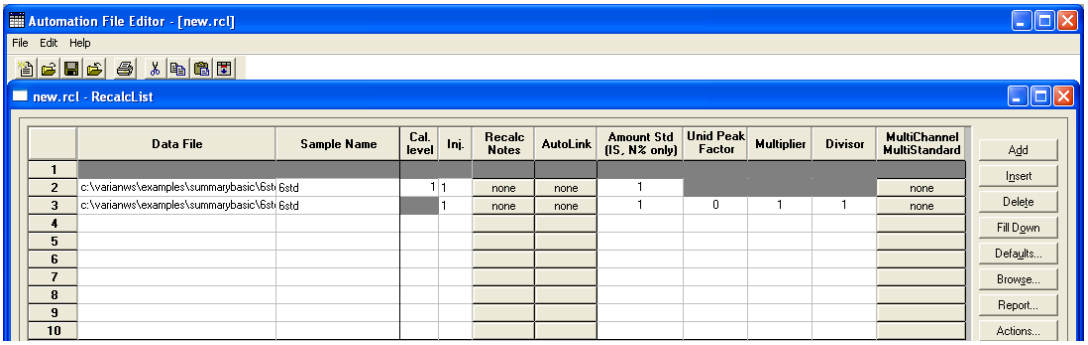

Notes - C:\VarianWS\Examples\SummaryBasic\new.rcl
These notes will be displayed in the Open File dialog box, and in automation file printouts.
OK
Cancel

Item	Description
Notepad Area	Shows notes attached to the file. The notes may describe the contents of the file, its uses, restrictions, etc. The Notepad Area is scrollable if the notes exceed the available space. The notes are edited here, either by typing text directly, or by pasting text from the Clipboard by pressing the keys: CTRL V simultaneously.

RecalcList Window

Add Data Files to a RecalcList by clicking a cell in the Data File column and typing the file name, or by clicking Brower and selecting the file name. To add one or more Data Files, quickly to a RecalcList is to select the files of interest in the Explorer and drag them to the RecalcList Window. When you release the mouse button, the Data Files are automatically appended to the RecalcList.

A RecalcList may contain standard LC data files (.run extension) and MS data files (.sms extension). Some the RecalcList items, described as follows, are handled differently for the two data file types. When the list is processed, the appropriate data handling is used automatically for each data file.



Item	Description
Data File	Shows the name and path of the Data File for each sample.
Sample Name	Cannot be edited. Shows the name of each sample in the RecalcList.
Sample Type	Baseline, Analysis, Calibration, Verification, Print Calib, New Calib Block, AutoLink, Activate Method Sets the sample type, or automation action, of each line in the RecalcList.
Cal. Level	1 to 10 Sets the calibration level of each calibration or verification sample in the RecalcList.
Inj	Cannot be edited. Shows the number of injections made of each sample in the RecalcList.
Recalc Notes...	Up to 180 characters Opens the Notes window for the selected record to edit or create a note about the sample. This note is stored separately from the original injection notes.
AutoLink...	There are two AutoLink commands when Sample Type is Baseline, Analysis, Calibration, or Verification. One AutoLink command when Sample Type is AutoLink. One Activate Method Command when Sample Type is Activate Method. Opens the AutoLink Parameters dialog box to set the options for linking to a remote application during automation through System Control. Opens the Activate Method Dialog Box to set the path name for activating a new method during automation of a RecalcList through System Control.
Amount Standard	0.000001 to 1,000,000.0 LC Files: Sets the amount of the first internal standard. Use to calibrate results for Internal Standard and Normalized Percent calculations. Not used by calibration samples. MS files: Sets an IS Factor used for Analysis and Verification samples. It is multiplied by the appropriate Compound Calibration Level Amount that is in the DH Method being used. Note that internal standards in Analysis and Verification samples use the amount specified in Calibration Level 1.
Unid Peak Factor	0 to 1,000,000.0 Sets a calibration factor for unidentified peaks. Not used for calibration samples.
Multiplier	0.000001 to 1,000,000.0 Sets a value for the multiplier. Results for the sample are multiplied by this value. Not used for calibration samples.
Divisor	0.000001 to 1,000,000.0 Sets a value for the divisor. Results for the sample are divided by this value. Not used for calibration samples.

Item	Description
MultiChannel MultiStandard	None, multiple, specific channel LC files: Opens the Data Handling Channels dialog box to specify the calibration parameters for up to four different Detector Channels. MS files: Not used for MS data handling.
Add	Adds a line to the end of the RecalcList.
Insert	Adds a new line before the highlighted line.
Delete	Deletes the highlighted row in the RecalcList.
Fill Down	Copies the contents of the top cell in a series of highlighted cells to the cells below it. Use to edit all the cells in a column quickly.
Default	Displays the Set Defaults dialog box, allowing you to specify default values for each applicable field in the RecalcList.
Browse	Opens the Open Data File dialog box to select a Data File to add to the RecalcList.
Report	Shows the results report for the selected Data File.
Actions	Displays a list of applications that can be opened with the selected data file, that is, Print Standard Report.
Begin	Opens the Begin RecalcList Dialog Box to specify a method to use while recalculating or printing all RUN files in this RecalcList, and to then begin recalculating or printing the files. Only available in the System Control version of the RecalcList.
Suspend	Suspends execution of this RecalcList after the current file is completed. Only available in the System Control version of the RecalcList.
Resume	Resumes execution of this RecalcList after it has been suspended. Only available in the System Control version of the RecalcList.

Data Handling Channels Dialog Box

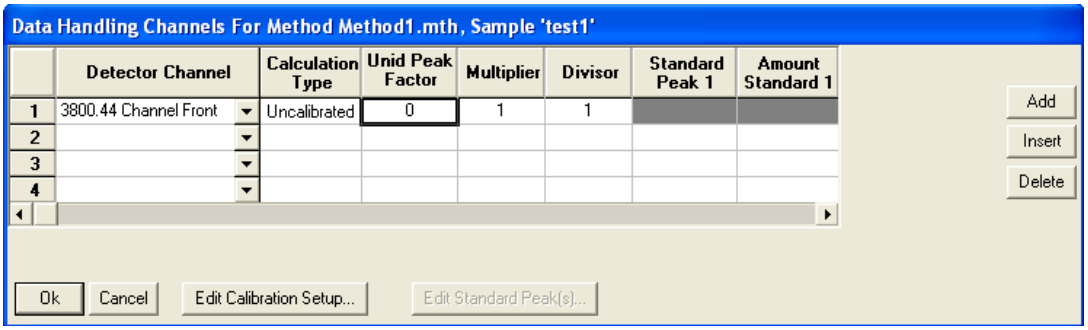
NOTE: Use this dialog box to specify standard LC data handling. MS data handling uses only one detector channel.

You can specify the Multiplier, Divisor, and Unidentified Peak Factor channel-by-channel for up to four different detector channels. And you can specify up to eight internal standards for each of the four channels, for a total of 32 internal standards.

NOTE: If you only want to specify a single Multiplier, Divisor, Unidentified Peak Factor, and Amount Standard to be used for all channels of all detectors. Use the SampleList, RecalcList, or Inject Single Sample dialog.

If you plan to specify parameters for each channel, before opening this dialog box make sure that the active method contains the data handling sections for each channel, and that their peak tables contain the standard peaks (if any) properly named and checked. The dialog box uses information from the active method to help you make the proper specifications for the type of calibration method that you are using.

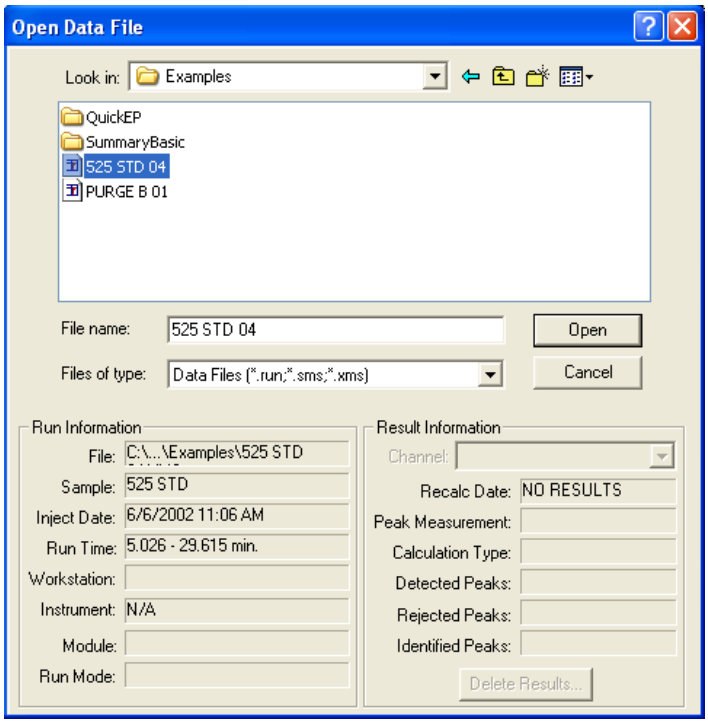
NOTE: If you change the standard peak names in the peak table, you must return to this dialog for each sample to update the names and amounts.



Item	Description
Detector Channel	Indicates the detector channel specified by the remaining fields in the row. Channels are indicated by the detector module name, bus address, and channel identifier. It has entries for all channels with data handling sections in the active method. If the desired channel is not visible, make sure that the correct method is active and that it contains data handling sections for the desired channel.
Calculation Type	Internal Std External Std Normalized % No Calibration Indicates the calibration method specified for the detector channel in the active method. To change the calibration method for a specific channel, click the channel's row, and then click **Edit Calibration Setup**. If the Calculation Type is not Internal Std or Normalized %, the "Edit Standard Peak(s)" push-button is disabled.
Unid Peak Factor	0 to 1,000,000 Set a calibration factor for unidentified peaks.
Multiplier	0.000001 to 1.000,000 Set a value for the multiplier. Results for the sample are multiplied by this value.
Divisor	0.000001 to 1,000,000 Set a value for the divisor. Results for the sample are divided by this value.
Standard Peak 1	40 character standard peak name from peak table Indicates the name of the first Internal Standard Peak. The Amount Standard 1 is applied to the Internal Standard Peak having the same name. To change the standard peak name for a specific channel, first click the channel's row, and then click **Edit Standard Peak(s)**.
Amount Standard 1	0.000001 to 1,000,000 Sets the amount of the first internal standard. Use to calibrate results for Internal Standard and Normalized Percent calculations.

Item	Description
Standard Peak 2-8	40 character standard peak name from peak table Indicates names of the multiple internal standard peaks present in the specific channel's peak table. To change the standard peak names for a specific channel first click the channel's row, and click Edit Standard Peak(s). If the peak table does not contain multiple internal standards, these fields are disabled.
Amount Standard 2-8	0.000001 to 1,000,000 Sets the amounts of the second through eighth internal standards. Use it to calibrate results for Internal Standard and Normalized Percent calculations involving multiple internal standards. If the peak table does not contain multiple internal standards, these fields are disabled.
Add	Adds a row to the end of the list.
Insert	Adds a new row before the highlighted line.
Delete	Deletes the highlighted row in the list.
Edit Calibration Setup	Opens Calibration Setup to permit inspection and editing of the calibration parameters in the method for the indicated detector channel.
Edit Standard Peak(s)	Opens Peak Table to permit inspection and editing of the Standard Peak names in the method for the indicated detector channel. Open the Peak Table and click **OK** to transfer the standard peak names and amounts from the Peak Table to the indicated detector channel row.

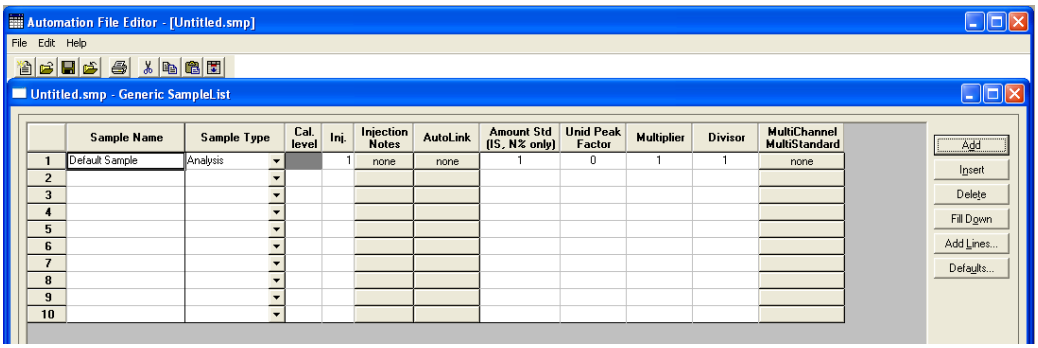
Open Data File Dialog Box



Item	Description
Look in	Lists the available folders and files. To see how the current folder fits in the hierarchy on your computer, click the down arrow. Click a folder to see the contents. The box above shows the folders and files in the selected location. You can also double-click a folder or file in that box to open it. To open the folder one level higher, click the up arrow button on the toolbar.
File list	Lists the folders and files in the selected location. Double-click a folder to see the contents. You can also use the Look In box to see the hierarchy of folders. To open the folder one level higher, click the up arrow button on the toolbar.
File name	Shows the currently selected file.
Files of type	Restricts the list of files to only those that match the selected.
Run Information	Shows information about the currently selected file.
Result Information	Shows information about the results, if any, calculated from the current data file.
Channel	Specifies the detector channel that will have Result Information displayed. Note that this applies only to standard LC results.
Delete Results	Delete results from currently select channel of the currently selected data file. This does not appear if disabled by the MS Workstation Security Administrator. NOTE: only affects standard LC results. MS results are not deleted.
Open	Opens the selected data file using the selected channel.
Cancel	Cancels file selection.

SampleList Window

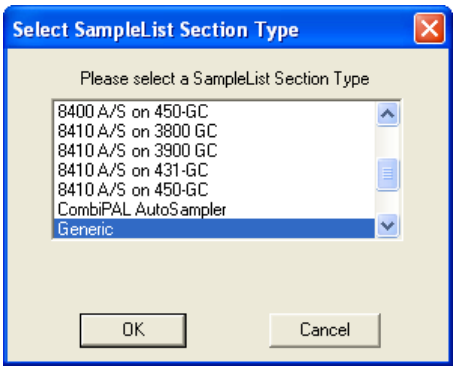
Contains injection parameters specific for your sample introduction device. This section describes the generic SampleList fields. Refer to the appropriate Instrument Control help topic in the System Control Reference Help for a description of the device-specific extensions to the SampleList.



Item	Description
Sample Name	Up to 19 characters Sets the name of each sample in the SampleList
Sample Type	Baseline, Analysis, Calibration, Verification, Print Calib, New Calib Block, AutoLink, Activate Method Sets the sample type, or automation action, of each line in the SampleList.
Cal. Level	1 to 10 Sets the calibration level of each calibration or verification sample in the SampleList.
Inj	1 to 9 Sets the number of injections to be made of the sample.
Injection Notes	Up to 180 characters Opens the Notes window for the selected sample to edit or create a note about the sample.
AutoLink	Two AutoLink commands when Sample Type is Baseline, Analysis, Calibration, or Verification. One AutoLink command when Sample Type is AutoLink. One Activate Method Command when Sample Type is Activate Method. Opens the AutoLink Parameters dialog box to set the options for linking to a remote application during automation through System Control. Opens the Activate Method dialog box to set the path name for activating a new method during automation of a SampleList through System Control.
Unid Peak Factor	0 to 1,000,000.0 Sets a calibration factor for unidentified peaks. Not used for calibration samples.

Item	Description
Multiplier	0.000001 to 1,000,000.0 Sets a value for the multiplier. Results for the sample are multiplied by this value. Not used for calibration samples.
Divisor	0.000001 to 1,000,000.0 Sets a value for the divisor. Results for the sample are divided by this value. Not used for calibration samples.
Amount Standard	0.000001 to 1,000,000.0 LC Files: Sets the amount of the first internal standard. Use to calibrate results for Internal Standard and Normalized Percent calculations. Not used for calibration samples. MS files: Sets an IS Factor used by Analysis and Verification samples. It is multiplied by the appropriate Compound Calibration Level Amount in the Data Handling Method used. Note that internal standards in Analysis and Verification samples always use the amount that is specified in Calibration Level 1.
MultiChannel MultiStandard	None, multiple, specific channel LC files: Opens the Data Handling Channels dialog box to specify the calibration parameters for up to four different Detector Channels. MS files: Not used by MS data handling.
Add	Adds a line to the end of the SampleList.
Insert	Adds a line before the highlighted line.
Delete	Deletes the highlighted line in the SampleList.
Fill Down	Copies the contents of the top cell in a series of highlighted cells to the cells below it. Use to edit all the cells in a column quickly.
Add Lines	Displays the Add Lines dialog box, allowing you to specify the number of lines to either insert or append to the spreadsheet, along with the values to use for each applicable field. Certain fields such as Sample ID and vial number can be automatically incremented.
Defaults	Displays the Set Defaults dialog box, allowing you to specify default values for each applicable field in the SampleList.
Data Files	Opens Data File Generation where you specify the naming scheme used for Data Files generated from injections. If the method contains both MS and standard LC DH method sections, then both sms and run files are generated.
RecalcList	Opens the RecalcList Generation dialog box to specify the options for generating or updating RecalcLists after injections. Note that if the method used both MS and standard LC Data Handling sections, then the generated RecalcList will contain both sms and run files.

Select SampleList Section Type Dialog Box

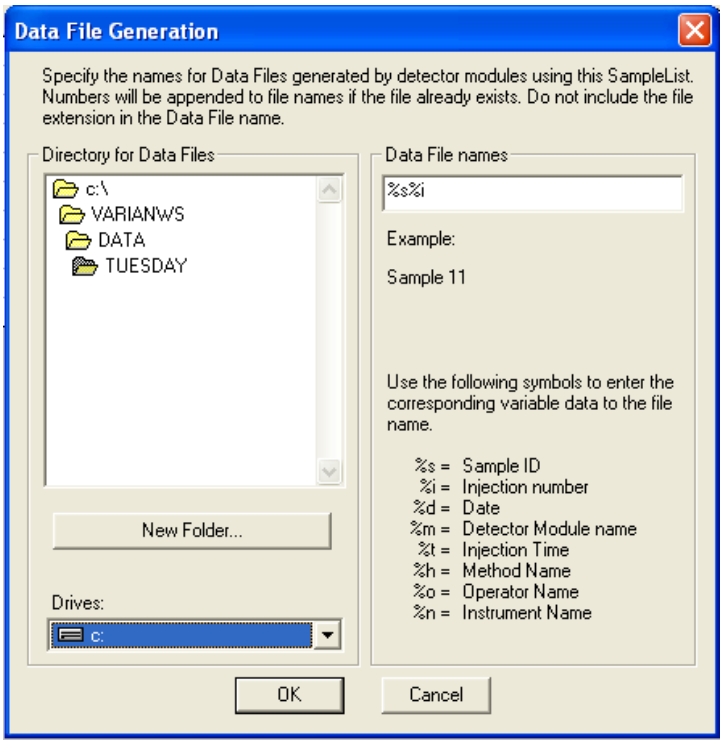


A SampleList File may contain multiple SampleList Sections. Each installed AutoSampler can contribute its own section, depending on the features that correspond to its hardware. Generally, each installed AutoSampler has its own unique SampleList with columns for its features. A SampleList File can contain a section for each type of AutoSampler, similar to the way that a Method file can contain a section for each type of Instrument Module.

This dialog box lets you select the specific section to edit in a SampleList file that may contain more than one section.

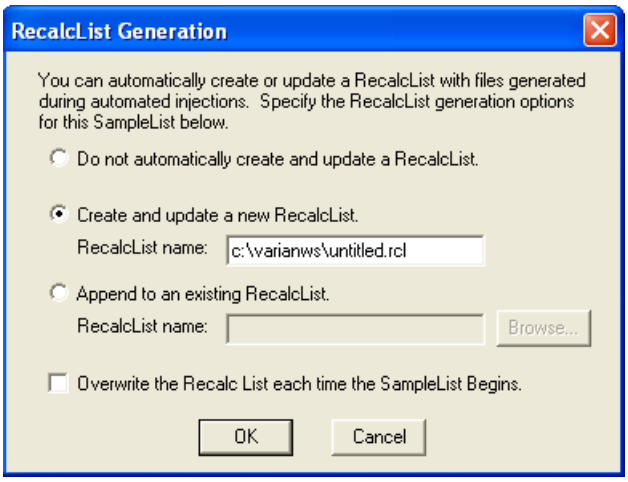
Item	Description
SampleList section type list box	Select a section type to use in building a new SampleList; opens the appropriate SampleList window. SampleLists may contain sections for multiple types of sample handling devices, depending upon which module drivers have been installed.
OK	Creates a SampleList section of the specified type.
Cancel	Cancels SampleList section creation.

Data File Generation Dialog Box


Data File Generation
Specify the names for Data Files generated by detector modules using this SampleList. Numbers will be appended to file names if the file already exists. Do not include the file extension in the Data File name.
Directory for Data Files
c:\
VARIANWS
DATA
TUESDAY
New Folder...
Drives:
c:
Data File names
%s%i
Example:
Sample 11
Use the following symbols to enter the corresponding variable data to the file name.
%s = Sample ID
%i = Injection number
%d = Date
%m = Detector Module name
%t = Injection Time
%h = Method Name
%o = Operator Name
%n = Instrument Name
OK
Cancel

Item	Description
Directory for Data Files	Shows the currently selected directory where Data Files are generated.
New Folder	Allows you to create and name a new folder in the currently displayed directory.
Drives	Allows you to specify the drive where Data Files are generated.
Data File Names	Allows you to enter the Data File name specification. You may enter as many characters as you wish, but the final file name (including path) must not exceed 255 characters in length. You may embed the special symbols listed in the window to represent sample-specific information in the Data File name. As you enter the specification, an example file name is shown.
OK	Accepts the name specification.
Cancel	Cancels the name specification.

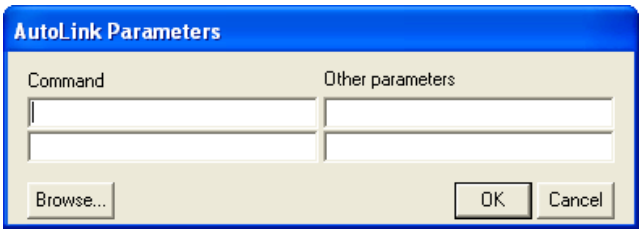
RecalcList Generation Dialog Box


RecalcList Generation
You can automatically create or update a RecalcList with files generated during automated injections. Specify the RecalcList generation options for this SampleList below.
Do not automatically create and update a RecalcList.
Create and update a new RecalcList.
RecalcList name:
c:\varianws\untitled.rcl
Append to an existing RecalcList.
RecalcList name:
Browse...
Overwrite the Recalc List each time the SampleList Begins.
OK
Cancel

Item	Description
Do not automatically create and update a RecalcList.	No RecalcList is generated or updated when you create Data Files as a result of injections.
Create and update a new RecalcList	Enter the name of a new RecalcList created when Data Files are generated as a result of injections. If the RecalcList name that you specify already exists when the injections are performed, a number is appended to the name so that it is unique.
Append to an existing RecalcList	Browse for an existing RecalcList file. When Data Files are generated as a result of injections, their information is appended to this file.
OK	Accepts the RecalcList specification.
Cancel	Cancels the RecalcList specification.

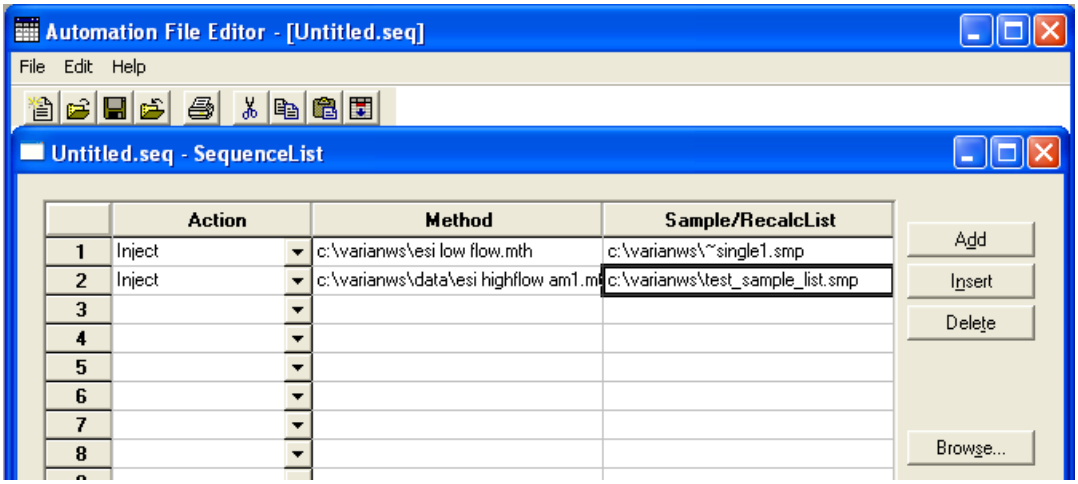
AutoLink Parameters Dialog Box

With AutoLink, you can specify a program to be executed after all injections of a sample have been performed, or two programs to be executed after each injection of that sample. When AutoLink appears as a sample type in a SampleList or RecalcList, the associated program is executed at that point in the SampleList / RecalcList after the previous SampleList / RecalcList line has completely finished. The AutoLink can be specified as part of a SampleList line that has a Baseline, Analysis, Calibration, or Verification sample type. In this case, the two AutoLink programs are executed after each injection of the sample.



Item	Description
Command	DOS command lines that open DDE applications Sets the command line used when AutoLink is invoked.
Other Parameters	Provides information to the remote application.
Browse	Opens the Select an AutoLink Program dialog box to select the name of an AutoLink program and enter it into the Command field.

SequenceList Window



Item	Description
Action	Shows the action chosen for each line in the Sequence.
Method	Shows the Method chosen for each line in the Sequence.
Sample/RecalcList	Shows the SampleList or RecalcList chosen for each line in the Sequence.
Add	Adds a new line to the end of the Sequence.
Insert	Adds a new line above the highlighted line.
Delete	Deletes the highlighted line in the Sequence.
Browse	Opens the Select a Method File or Select a Sample/RecalcList window to browse through directories and find a Method or Sample/RecalcList file to add to the Sequence.